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ЭТОТ НОМЕР ЖУРНАЛА ПОСВЯЩАЕТСЯ 50-ЛЕТНЕМУ ЮБИЛЕЮ АКАД. **ИВАНА МАЛЕКА**

DIESES HEFT IST DEM AKADEMIKER **IVAN MÁLEK** ANLÄSSLICH SEINES 50. GEBURTSTAGES GEWIDMET

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28-го сентября 1959 г. исполнится 50 лет академику Ивану Малеку, почетному члену Чехословацкой Академии Наук. Можно смело утверждать, и это не будут пустые слова, что юбиляр находится в полном расцвете физических и духовных сил. Одухотворенный истинным оптимизмом настоящего ученого, он отдает всю свою энергию служению науке, своей Родине и, можно сказать, всему человечеству. Как врач и биолог он никогда не забывал, что прежде всего должен служить жизни, и поэтому в своих многочисленных выдающихся теоретических работах он никогда не умалял практического значения науки. Уже его внешний облик свидетельствует о его исключительной жизненной энергии и трудоспособности. Искренний по натуре он всегда готов помочь там где это необходимо для успеха науки.

Профессор, доктор биологических наук Иван Малек родился в Забржеге на Мораве; по окончании медицинского факультета Карлова Университета в Праге он остался работать в качестве ассистента. В 1945 г. он защитил диссертацию по медицинской микробиологии и с 1948 по 1951 г. был доцентом на медицинском факультете в Градце Кралове. Уже в это время он организовывал новый центральный биологический институт, из которого позднее образовался Биологический институт Чехословацкой Академии Наук. В 1952 г. он стал членом Чехословацкой Академии Наук, где был избран председателем биологического отделения.

Под его руководством Биологический институт вырос в большое научное учреждение с широко развернутой многостороенней деятельностью, о котором смело можно сказать, что оно было создано Иваном Малеком. В нем находятся почти все отделы биологии, расчленные в специальные отделения, где работает 140 научных работников под руководством ведущих специалистов. Превосходное современное оборудование лабораторий и широкая поддержка со стороны правительства являются заслугой директора института профессора И. Малека. Неутомимо поддерживает он своих сотрудников при печатании научно-исследовательских работ; именно ему обязаны мы выход в свет настоящего журнала.

Колоссальный политический и социальный переворот, который до основания переменил жизнь Чехословакии, нашел в лице академика Малека горячего борца за новый мир.

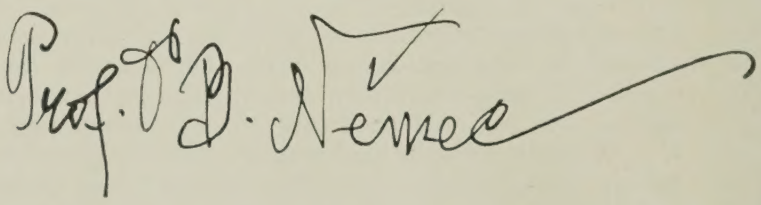
Профессор Малек находится в курсе научных исследований других государств. Во время длительного пребывания в Соединенных Штатах Америки он изучил там микробиологию и выполнил несколько научных работ в различных университетах. Прежде всего, свой опыт и здания он обогатил знанием богатого фонда советской науки. Он часто бывал за границей, и было им организовано много научных конгрессов при участии иностранных ученых.

Поддерживая традиции миролюбивых взглядов Я. Коменского, Лейбница, Канта и Е. Пуркинье, он всегда был горячим пропагандистом международного движения за мир. В качестве члена передовой организации Всемирной федерации научных работников (основанной Фредериком Жолио-Кюри, Джоном Берналом и другими) он организовал в Праге бюро для стран средней и восточной Европы, которое играет большую роль в развитии этой передовой организации ученых, объединяющей в настоящее время более 220 000 членов. Избрание членом иностранных и чехословацких научных академий и обществ, а также особые почетные награды в ЧСР являются высоким признанием его заслуг.

Более 100 опубликованных научных трудов свидетельствуют об его интенсивной научной деятельности. Особенно значительными и оригинальными были его опыты с культивированием микроорганизмов в жидких континуальных средах; также его исследования в области размножения бактерий, и, главным образом, открытие неравноценности дочерних клеток при размножении бактерий заслуживают самого серьезного внимания.

Кроме этих работ им были опубликованы многочисленные монографии учебники, популярные статьи и книги, так же как и современные методические указания.

Пожелаем же нашему юбиляру еще много лет дальнейшей плодотворной научной, организационной и общественной деятельности.



Zum 50. Geburtstag von Professor Dr. Ivan Málek

Am 28. September 1959 begeht Professor Dr. Ivan Málek, Mitglied der Akademie, seinen 50. Geburstag. Es ist keine Phrase, wenn ich sage, dass er ihn in voller Lebens- und Geisteskraft begeht, der jener frohe Optimismus eines Wissenschaftlers entspricht, der seine ganze Energie in den Dienst der Wissenschaft, in den Dienst seines Staates und man darf ruhig behaupten der ganzen Menschheit gestellt hat. Als Arzt und Biologe hat er nie vergessen, dass er dem Leben dienen soll und bei seinen zahlreichen bedeutenden theoretischen Forschungsergebnissen hat er die praktische Bedeutung der Wissenschaft nie unterschätzt. Man kann seine Lebensenergie, seine Tatkraft und seine Begeisterungsfähigkeit schon aus seinem Antlitz lesen; persönlich ist er ohne Hinterlist und immer bereit, seinen Einfluss für die Wissenschaft einzusetzen.

Geboren in Zábřeh in Mähren, studierte er an der Karlsuniversität in Prag Medizin, an der er nach Vollendung seiner Studienjahre Assistent wurde. Im Jahre 1945 habilitierte er sich für medizinische Mikrobiologie und dozierte vom Jahre 1948 bis 1951 an der medizinischen Fakultät in Hradec Králové. Bereits in dieser Zeit organisierte er das neugegründete Zentralinstitut für Biologie, aus dem sich bald nach der Gründung das Biologische Institut der tschechoslovakischen Akademie der Wissenschaften bildete. 1952 wurde er zum Akademiemitglied ernannt und die biologische Sektion der ČSAV wählte ihn zu ihrem Vorsitzenden.

Unter seiner Leitung ist das biologische Institut zu einer grossen, vielseitig tätigen Anstalt herangewachsen, die Máleks Schöpfung genannt werden muss. Es sind fast alle Fächer der Biologie vertreten, in spezielle Abteilungen eingeordnet und werden von etwa 140 wissenschaftlichen Kräften unter Fachvorständen bearbeitet. Die vorzügliche, moderne Ausstattung der Abteilungen und ihre ausreichende Unterstützung durch die Regierung verdankt das Institut seinem Direktor Professor Málek. Unermüdlich unterstützt er seine Mitarbeiter bei der Veröffentlichung ihrer wissenschaftlichen Resultate. Wir verdanken die Bewilligung der vorliegenden Zeitschrift (Biologia plantarum) ebenfalls seinen Bemühungen.

Der grosse politische und soziale Umbruch, der das Leben des tschechoslowakischen Staates von Grund auf umgeändert hat, fand in Akademiemitglied Dr. Málek einen der eifrigsten Vorkämpfer der neuen Welt.

Introduction

The measurement of leaf area plays a direct and important part of research in photosynthesis and related questions. Leaf area is also an important factor in the study of the water relations and nutrition of plants, in examining the influence of herbicides and stimulators and in investigating the mutual relations between plants. In such cases it is often necessary to measure large numbers of leaves and for this reason attempts are made to accelerate the operation as much as possible.

Very rapid and sufficiently accurate measurement of leaf area is possible by photometric methods in which the reduction in light flux caused by the object to be measured makes it possible to deduce its area. Besides visual photoplanimeters (e. g. Bolas and Melville 1933), a number of photoelectric planimeters have been suggested, based on various principles and having the most various constructions (e. g. Spoehr and McGee 1923, Gerdel and Salter 1928, Frear 1935, Petrov and Gavrilov 1939, Grigor'ev and Metreveli 1958). The great majority of these instruments suffer, however, from the drawback that the data they afford are not dependent only on the area of the objects measured, but also to a greater or lesser extent on their optical properties. In the case of leaves, their thickness and colour, and the proportion of light which they reflect are important. Such instruments have to be calibrated using samples of known area, taken from leaves of the same species as those to be measured. In the case of articulated leaves such calibration is difficult, if not impossible.

This drawback is removed in principle in the apparatus described by Miller, Shadbolt and Holm (1956). Its construction, however, requires good quality large Fresnel lenses, which are not always available. It is therefore necessary to seek another solution, which reduces the effect of the optical properties of the leaves, to a minimum.

In the apparatus described here data sufficiently independent of the quality of the leaves measured are obtained by following the absorption of rays within the wave lengths of 0·6 to 1·4 μ, that is within a range where the leaves absorb radiation to a great extent. In addition, such weak illumination is used that almost total absorption is achieved. Independence of the readings of this apparatus on fluctuations in the output of the source of radiation is obtained using a balanced circuit with two AgS-photovoltaic cells. For the detection of the differences in the very weak photoelectric currents from these photoelectric cells a very sensitive and highly stable amplifier is used.

Construction of the Instrument

Fig. 1 shows the block scheme of the instrument, divided into two identical channels. The flux of radiation from a plane source S passes the filters F′ and F″ in each channel and is further directed by the two light leading

prisms C_1 and C_2 to the photocells P_1 and P_2. The object O whose area is to be measured is placed between the filters in one of the channels. The resultant difference in the photoelectric currents is amplified by means of a differential amplifier (A) and indicated by the galvanometer (M).

Fig. 2 gives a photograph of all operational parts of the instrument.

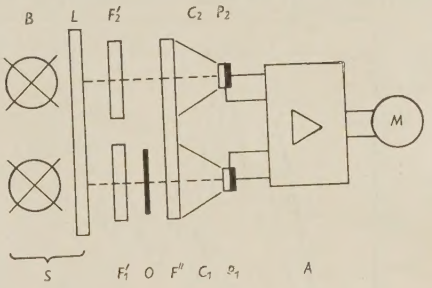


Fig. 1. Block scheme of optical planimeter. For explanation see text.

Optical parts. The source of radiation consists of four regularly spaced glow lamps 127 V/26 W, two above each channel. In view of the spectral properties of the filters and photoelements used, it is preferable to operate them at a lower than nominal voltage, e. g. at cca 70 to 80 volts. The differential method of measurement makes the stabilization of the feeding voltage superfluous. A sheet of milky lucite 6 mm. thick placed beneath the bulbs scatters the light and makes the iradiation of the working area of both channels reasonably even.

The leavers are placed on a 2 mm. sheet of getinax in the measuring channel and are covered by a sheet of red-coloured lucite 4 mm. in thickness. Getinax

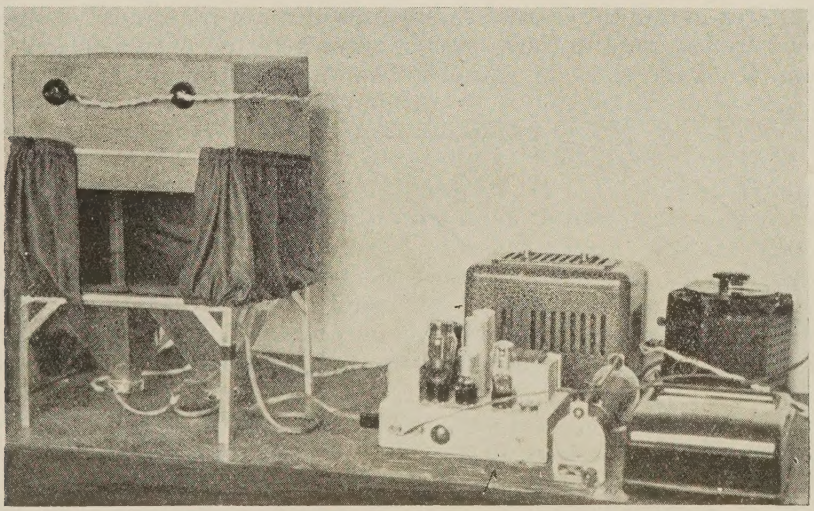


Fig. 2. General view of the planimeter

is a plastic produced by the impregnation of paper pulp by bakelite. Therefore it is not transparent, it scatters light and absorbs a great deal of the radiation falling on it; only radiation of $\lambda > 0{\cdot}6\ \mu$ is transmitted (v. Fig.3). The spectrum of the radiation reaching the channels is further modified by the coloured lucite mentioned above, which was obtained by staining clear

lucite with fuchsin ("Magenta-Rot" Merck preparation). Transmission spectrum of the filter formed by this combination of getinax and red lucite shown in Fig. 3, which also includes a diagram of the spectral characteristics of the photovoltaic cells used.

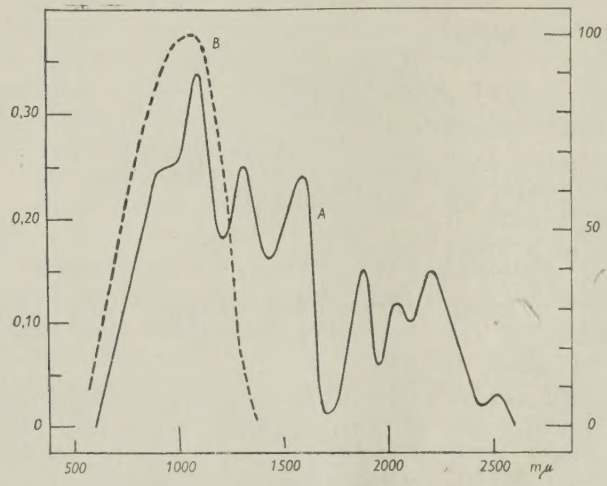


Fig. 3. Transmission spectrum of combined filters F′ and F″ (curve A) and spectral characteristics of the argentum sulphide photocell (curve B). Abscissa: Wave length in mμ. Ordinate (left): transmission in % (curve A), ordinate (right): selective spectral sensitivity in % (curve B).

The working area in each channel is 34 × 28 cm². The flux of radiation from this area enters the optical channels, which are pyramidal in shape. The photocells are mounted in the apexes of these inverted pyramids at a distance of 25 cm. from the sheet of getinax. The channels are made of sheet brass

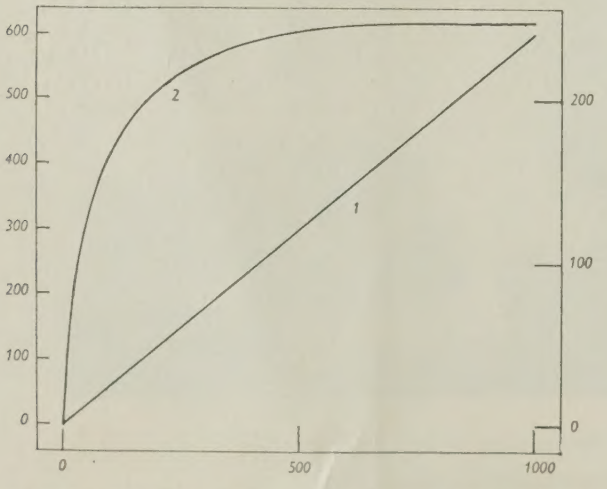


Fig. 4. Photoelectric characteristics of the argentum sulphide photocell. Abscissa: Illumination in lux. Ordinate (left): Short-circuit current in μA (curve 1), ordinate (right): opencircuit voltage in mV (curve 2).

and are lined by a layer of white material which scatters light. This is prepared by painting the insides of the channel walls with lacquer, which is covered by a layer of fine MgO or $BaSO_4$ powder before it has dried. The surplus powder is blown off after the lacquer has dried. Optical channels constructed in this way do not completely eliminate the influence of the position of the

object in the measuring field. The objects are, therefore, always placed as far as possible in the centre of this field, which is marked by a cross.

Barrier-layer photovoltaic cells, with argentum sulphide as semiconductor, are used for the detection of radiation. These were constructed at the Institute

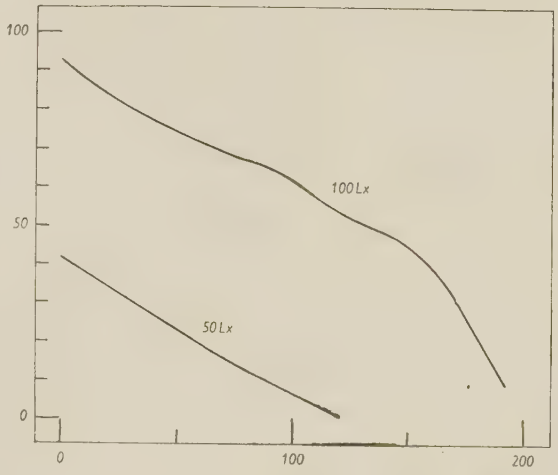


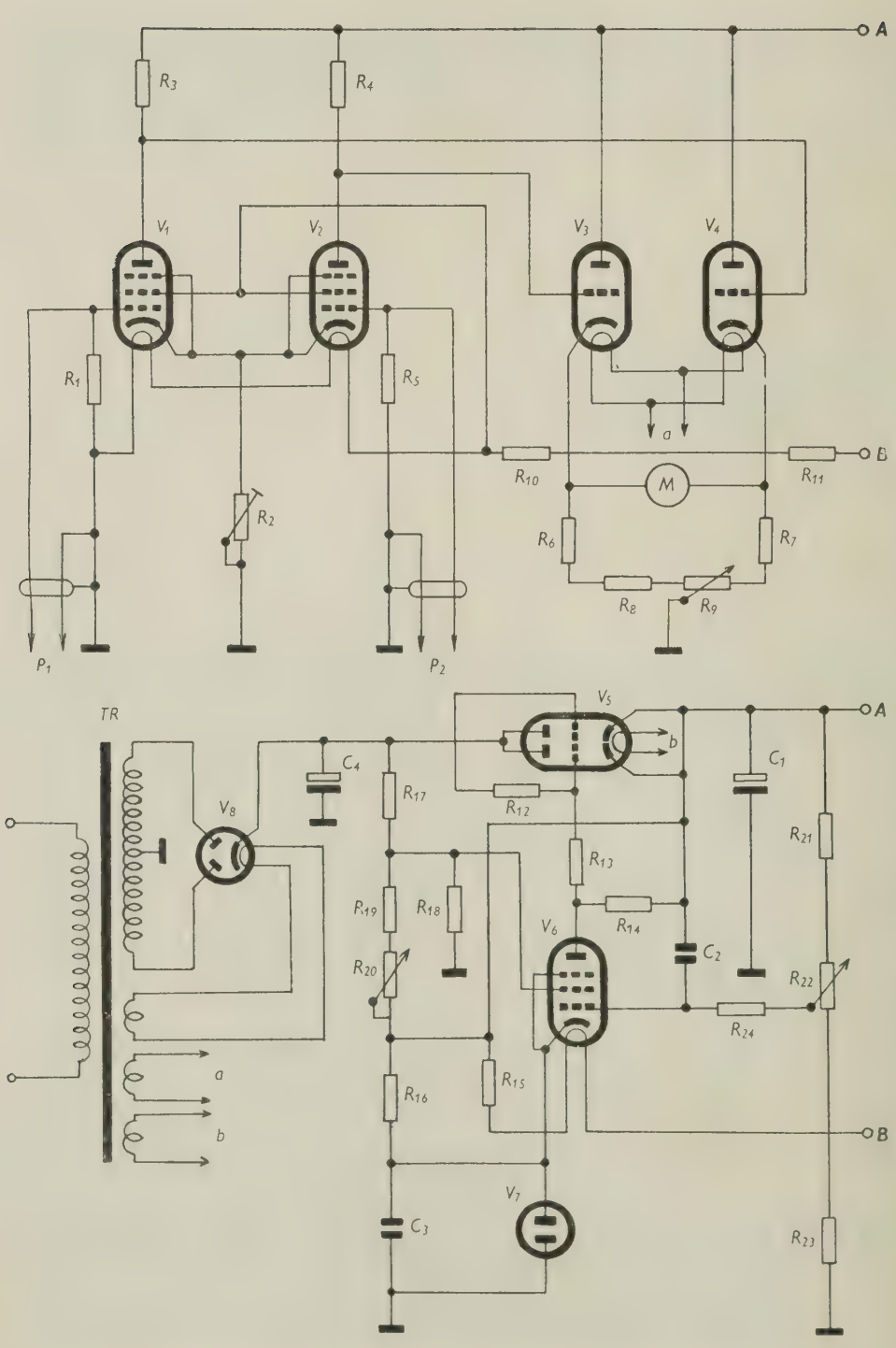
Fig. 5. Voltampere characteristics of the AgS photocell. Abscissa: Output voltage in mV. Ordinate: photoelectric current in μA.

of Physics of the Ukrainian Academy of Sciences. As is shown in Fig. 3, the transmission spectrum of the filters corresponds very well with the spectral sensitivity curve of the photovoltaic cells. The photoelectric characteristics of the photocells used, with a cross section of 2 cm^2, are given in Fig. 4. The voltampere characteristics of the cells are shown in Fig. 5. As can be seen from this figure, with 50 lx the relationship is still linear, but with higher irradiation intensities it deviates from linearity. It is therefore advantageous that in the instrument described only very weak radiation falls on the photovoltaic cells. Temperature dependence of short-circuit current and open-circuit voltage is presented in Fig. 5. Internal resistance of the photocells varies between 1,000 and 6,000 ohms. The photocurrent at a given intensity of radiation changes with time during the first two months during which the photocells are in use; in the case of output voltage the period is longer (5 to 6 months).

T h e a m p l i f i e r. The difference in the photocurrents coming from the measuring and control photocell is amplified by means of a two-stage differential d.-c. amplifier (v. Fig. 6). The voltage generated by the photocells across the load resistance of several hundred kiloohms is supplied to the grids of the tubes in the first balanced pentode stage (V_1 and V_2). The load resistors of the first stage were experimentally chosen so as to balance the deviations existing between the photovoltaic cells P_1 and P_2. Gain in the first stage may be regulated by means of the variable resistor R_2. In this way it is possible to get a suitable reading of the meter for a given area.

The second cascade (V_1 and V_2) provides a low output impedance. The bridge connection again guarantees independence of fluctuations in the supply.

To make the drift of the amplifier as low as possible, the supply voltages


A
R3
R4
V1
V2
V3
V4
R1
R5
a
B
R10
M
R11
R2
R6
R7
R8
R9
P1
P2
TR
V5
b
C4
R17
R12
C1
V8
R21
R13
R19
R18
R14
V6
C2
R20
R22
R24
a
R16
R15
B
b
V7
C3
R23

for both stages and the heating current for the tubes of the first stage are provided with an efficient electronic voltage stabilizer. In addition the heating current of tubes V_1 and V_2 is stabilized by the resistors R_{10}, R_{11} and R_{15}.

The electronic stabilizer is governed by the error of the output voltage (voltage divider R_{21} to R_{23}, control grid of tube V_6) and by the error of unstabilized input voltage through the compensating circuit (voltage divider $R_{16} + R_{20}$, screen grid of V_6). Constant reference voltage is provided by the glow-tube, operating at 75 volts. Heating current of the amplifier (V_6) is derived from the stabilized supply. The stabilizer described delivers the voltage necessary for operation of the amplifier with a high stability (stabilization coefficient at about 2,000) if the input voltage varies between —20 to +10%.

The power supply is of normal type with a full-wave rectifier (V_8).

Putting the instrument into operation. Firstly, it is necessary to find the proper operation point of the stabilizer. For this purpose the amplifier is disconnected at points A and B and is replaced by a variable resistor (R_S — 600 ohms); suitable measuring instruments are connected as shown in Fig. 7. The variable resistor R_{21} is adjusted to approximately its middle position. The output voltage of the stabilizer is brought to 162 to 164 volts by adjusting the potentiometer R_{24} and the output current is adjusted to 72 to 75 milliamperes varying the voltage of the substitute load R_S. Input voltage is then varied by means of a variable voltage transformer between the limits of —20 to +10% from the normal mains voltage and the meter V_2, connected in series with a compensating battery, is read. The resistance of the variable resistor R_{21} is progressively varied (with simultaneous correction of output voltage through adjustment of the potentiometer R_{24}) until a state is reached in which the variations of input voltage exercise a minimal effect on the output voltage of the stabilizer.

← Fig. 6. Diagram of the electronic part of the instrument. Scheme is drawn corresponding to the Czechoslovak norms.

R_1	430 kΩ/0,25 W	R_{15}	800 Ω/50 W	V_1, V_2, V_6	— 12 ЖЛ1П may be substitued e. g. by RV 12 P 2000
R_2	6·8 kΩ	R_{16}	16 kΩ/2 W	V_3, V_4	— 6C65 could be substitued by 6SN7 or E CC 40 etc.
R_3	180 kΩ/1 W	R_{17}	1·3 MΩ/0,25 W	V_5	— 6H5C a valve of rather solitary construction; might be substitued by two valves of the type PL 36 connected as triodes.
R_4	240 kΩ/1W	R_{18}	47 kΩ/1 W		
R_5	430 kΩ/0,25 W	R_{19}	52·6 kΩ/1 W		
R_6	43 kΩ/1 W	R_{20}	10 kΩ		
R_7	51 kΩ/1 W	R_{21}	106 kΩ/1 W	V_7	— CT'2C equivalent to VR 75 or any other glow tube with Vstab. = 75 V, $I_{max.}$ = 40 mA, $I_{min.}$ = 5 mA, $R_{i\,max.}$ = = 180 Ω.
R_8	100 kΩ	R_{22}	4·7 kΩ		
R_9	47 kΩ	R_{23}	82 kΩ/1 W		
R_{10}	800 Ω/50 W	R_{24}	560 kΩ/0,25 W		
R_{11}	75 Ω/15 W				
R_{12}	330 Ω/0,25 W	C_1	40 μF	V_8	— 5 Ц4 C equivalent to 5Z4.
R_{13}	330 Ω/0,25 W	C_2	9100 pF		
R_{14}	10 MΩ/2 W	C_3	0,07 μF		
		C_4	40 μF		

V_1—V_8 see text

P_1—P_2 to photoelements

The amplifier with the photocells is then connected. The variable resistors R_8 and R_9 are adjusted in approximately their middle position. With weak illumination of the photocells, R_3 and R_4 are experimentally adjusted so as to bring the deflection of the meter near zero. Finally, residual asymmetry, zero position and gain are adjusted by means of R_8, R_9 and R_2 respectively. The gain of the amplifier is chosen so as to bring direct readings of the meter into simple relation to the area of the objects measured (e. g. when 2 cm² corresponds to 20 divisions of the scale).

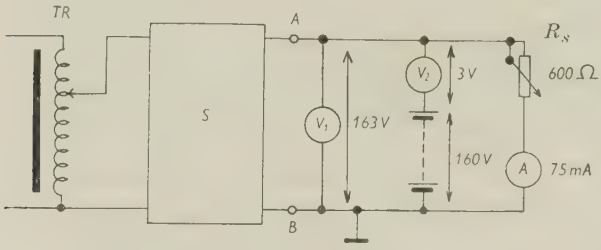


Fig. 7. Circuit for adjusting the voltage stabilizer.

Calibration of the instrument. Calibration is carried out by inserting pieces of black paper (circular or square) with area of 1 to 150 cm² into the measuring channel and noting the readings of the meter. An advantage of the instrument is that it is possible to choose the gain of the amplifier so that the maximum area measured would cause a full deviation of the mater. i. e. a unit of area will correspond to a definite number of divisions.

Another method of measurement can be adopted, reminiscent of weighing on an ordinary balance: the objects whose area is to be measured are placed in one channel and round or square pieces of black paper of known area are placed in the other until the meter returns to zero.

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Прибор для измерения площади листьев

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Резюме

В статье авторы описывают новый тип фотоэлектрического планиметра для измерения площади листьев. Планиметр основан на абсорбции крайнего красного и близкого инфракрасного излучений, и поэтому его показания мало зависят от толщины и окраски листьев. Конструкция оптической части аппарата довольно проста. В планиметре имеются два оптических канала: один измерительный, в который вкладываются листья, и другой, контрольный (эталонный), который компенсирует колебания в силе потока света, вызываемые изменениями напряжения в сети. Поток света в обоих каналах переменяется в электрические импульсы при помощи серебряносульфидных фотоэлементов. Различие между напряжением измерительного и эталонного фотоэлементов усиливаются при помощи чувствительного дифференциального усилителя постоянного тока, собранного по балланснои схеме. Для обеспечения стабильности нуля все анодные цепи этого усилителя и накальные цепи первого каскада питаются от электронного стабилизатора. Коэффициент усиления можно менять и тем самым легко можно установить цену деления шкалы прибора. Сходное устройство обоих каналов позволяет, кроме прочего, применить метод измерения, аналогичный взвешиванию на весах: в контрольный канал вкладываются квадраты или кружки с известной площадью, пока указатель прибора не находится на нуле. При помощи планиметра нельзя измерять большое количество объектов одновременно, так как ось канала должна проходить через измеряемый объект.

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The Influence of Photoperiodicity on the Sexual Index in Hemp (*Cannabis sativa* L.)

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Souhrn

1. Vysévali jsme konopí (*Cannabis sativa* L.) postupně v měsíčních intervalech od listopadu do srpna v letech 1952/53 a 1953/54. U jednotlivých výsevů byla u samčích a samičích rostlin sledována doba do květu. Samčí rostliny kvetou zpravidla dříve než rostliny samičí. Při prodlužujícím se dnu (nad 14 hod.) přestanou samičí rostliny kvést (pokud nekvetly v tuto dobu, nerozkvetou) a pokračují jen ve vzrůstu. Při silně ubývajícím dnu (den kratší než 15 hod.) počnou kvést a jejich doba do květu se tak prodlužuje až na 265 dnů. Jsou to vždy rostliny samičí. Rostliny samčí kvetou i při dnu delším než 14 hod. Jsou tedy fotoperiodicky rostlinami neutrálními s tendencí být rostlinami dlouhého dne. Rostliny samičí jsou rostlinami krátkého, lépe řečeno ubývajícího dne. Nejmenší podíl samčích rostlin je ve výsevech nejkratšího dne.

2. V polních podmínkách jsme v letech 1953/54 provedli zastiňování mladých rostlin konopí v různých fázích růstu. Ukázalo se, že snižování světelné intensity zvyšuje podíl samičích rostlin a to zejména, trvalo-li zastiňování 14 až 21 dnů a začalo-li v roce 1953 do 14 dnů a v roce 1954 do 7 dnů od vzcházení. Různé výsledky z obou let vysvětlujeme různými klimatickými podmínkami.

3. Za určitých podmínek se vyskytují u konopí též rostliny intersexuální. V našich pokusech byl jejich výskyt vázán na velmi krátký den (11 až 13 hod.) a vyskytovaly se v množství 22—30 %. Považujeme intersexuální rostliny za rostliny samčí, u nichž vliv krátkého dne nedovolil čistou pohlavní diferenciaci. Ačkoliv se tedy tyto rostliny vyskytují pouze při extrémně krátkých fotoperiodách (optimálních pro rostliny samičí), je jejich počet dnů do květu přibližně střední mezi počtem dnů potřebných pro rostliny samčí a samičí. Po trojnásobné opakované dekapitaci intersexuálních rostlin se při dosažení potřebné délky dne vyvinuly tyto rostliny v rostliny samčí.

4. Roubováním různě pohlavních rostlin nebylo dosaženo žádné změny pohlaví, pravděpodobně pro krátké trvání vegetativního sblížení.

Summary

Hemp (*Cannabis sativa* L.) was sown at intervals from November to August. For each sowing the time of flowering was recorded for male and female plants separately. Male plants usually flowered earlier than female. When the period of daylight exceeds 14 hours, that is when the days are lengthening in April, the female plants stop flowering and continue their vegetative growth. They start flowering again when the days are shortening considerably, in August, when the daylight period is about 15 hours. The male plants flower even when the day is longer than 14 hours.

In experiments during which the plants were shaded it was found that reduction of light intensity leads to an increase in the number of female plants, particularly if shading is carried out in the early stages of the plant's development.

The occurrence of intersexual plants was observed only during the short day period (11 to 13 hours), when they composed 22—30% of the total. When the day exceeds 13 hours intersexual plants no longer develop.

The grafting of plants of varying sex did not produce any sexual change—probably due to the fact that the period of vegetative approximation was short.

Introduction

Hemp is a dioecious plant with a sharp sexual dimorphism. A very small proportion of monoecious plants occur (these are in fact female plants with a small number of male flowers). Their monoecious character is, however, very unstable and in order to maintain it constant selection is necessary (Arinshteyn and Senchenko 1956). Under quite exceptional conditions intersexual plants can occur, in which case they are abundant.

Hemp has for a long time been the classical material for experiments on the sexuality of dioecious plants.

The question of whether or not male and female sex of dioecious plants is already determined in the embryo has been on the whole settled by Schaffner's (1921) extensive work, which gives a clearly negative result.

Similar conclusions from work with hemp (Lyubich 1950) and European hop (*Humulus lupulus* L.) indicate that both sexes are already established in the seed (Osvald and Blattný 1954). The determination of sex in young plants is still in the experimental stage (Kulik 1953).

The fact that the sexual index of hemp changes under the influence of environmental conditions has been well known and fully documented. It has not, however, been sufficiently clear which components of the environment are the most decisive in determining the sexual index, how they act in the formation of sexual polarity and at which period of ontogenesis their intervention is necessary for them to be effective (Borthwick and Scully 1954) Matusiewicz (1953) has found that when the day was artificially shortened (10 hours) hemp flowered after 67 days, while the control plants flowered after

127 days. He does not mention whether the plants were male or female. He followed the growth of male and female plants with short and long days. When the day was shortened to 10 to 8 hours intersexual plants also occurred.

In the present experiments the influence of light intensity and day length has been the chief subject of study.

Results and Discussion

Ia. The flowering period of male and female plants in relation to photoperiodism

In 1952 and 1954 sowing was carried out at monthly intervals from November to August. The seed was sown in boxes in the glasshouse. The total number of plants in the experiment was 6,152 with 1,333 and 4,819 in the different series. The variety of hemp used was "Jihoslovenská krajová". The soil and watering was the same for all plants. They sprouted evenly, on an average 8 days after sowing.

The flowering period was counted when about 30% of the plants began to flower, the remainder flowered within two further days. Results are given in graphs 1 and 2.

For ♀ plants the period of vegetation up to the start of flowering was often very different for the different sowings. For ♂ plants the length of the vegetative period up to the time of flowering was less influenced by the external environment. As a rule ♂ plants flower earlier than ♀. In some sowings ♂ plants flowered later than ♀.

It is clear from the experiments that for the formation of flowers and flowering of ♀ plants rapidly lengthening days are not favourable. When in April the day exceeds 14 hours ♀ plants cease flowering. They only continue to grow, without regard to age. Only when the day is considerably shortening in August, with a day length of about 15 hours, the temporarily non-flowering ♀ plants start to flower again.

The ♂ plants of hemp sown in November start to flower on 21 March, i. e. after 126 days, and flowering is 100%. Female plants start to flower on March 3, that is after 108 days. Some of them stop growing until in June the lower nodes send out new shoots. The foundations of flowers are formed on them, but flowering starts on August 8, i. e. 265 days after sowing. Flowering was "postponed" in this way in 22·1% of the female plants.

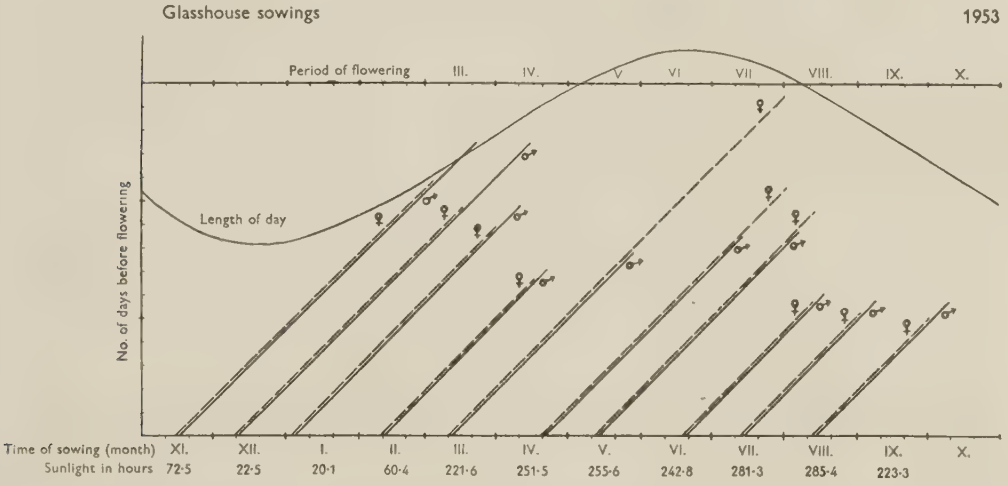
"Postponement" of flowering was repeated in further sowings up to the February sowing. This is only a quantitative difference. Plants from these sowings, insofar as they did not flower, continued to grow, did not send out auxiliary shoots, but flowered in August on their main axes. The majority of the female plants flowered within 67 days, later plants in 186 days.

In the case of the March and April sowings, plants from which should have flowered in 80 days in May to June, the female plants developed only "vegetatively" and began to flower as did some of the February-sown plants—after 145 days, i. e. in August when the day was shortening to 15 hours.

The male plants from the February sowing flowered in April after 72 days and from the March sowing in May after 79 days.

Thus the female plants in the glasshouse did not flower from the middle of April to the end of July, i. e. when the day was longer than 14 hours. They are, therefore, s h o r t - d a y photoperiodic plants.

The male plants flowered without almost any delay even when the day was longer than 14 hours, i. e. in May, June and July as well. They are, therefore, n e u t r a l photoperiodic plants, w i t h a t e n d e n c y t o w a r d s

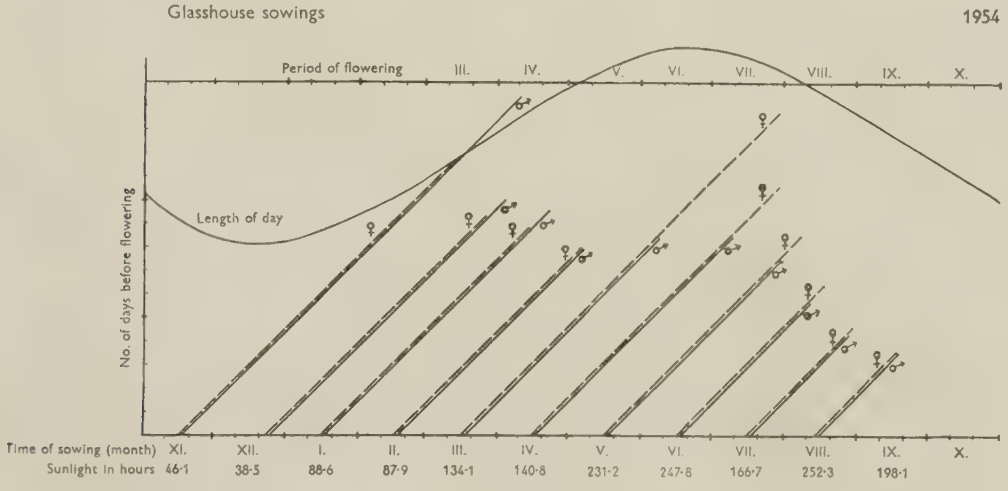


Explanation of graphs

The ordinate represents the period before flowering, 1 section = 10 days. The lower abscissa shows the month and day of sowing. 1 section = 10 days.

The second line shows the period of sunlight in hours for each month. The upper axis shows the day and month of flowering. (The curve shows day length in hours.)

Graphs 1 a 2 show clearly that male plants do not always flower earlier than female.



being long-day plants (the smallest number of male plants was to be found in the November to January sowings, when there are 8—10 hour days).

In the case of the May, June and July sowings female plants flowered later than the male, but as compared with earlier sowings they considerably shortened the time required before coming to flower. Female plants from the August sowing needed 50 days before flowering in 1953 and only 36 days in 1954. In 1954 there was less sunshine than during the same period in 1953.

The influence of a too-short day and definitely also the reduction in daylight intensity started to be effective in plants sown in November, December, January and February. Female plants from the September sowing flowered on November 4, after 57 days. Intersexual plants began to appear in this sowing. The occurrence of intersexual plants is particularly typical for plants from the November, December, January and February sowings. Hemp sown in September and October always perished in the winter. The sexual index of plants sown in November, December, January and February changes in favour of female plants: there is a greater number of female plants than male; with high light intensity the index is nearly 1 : 1. This corresponds to the results of experiments involving the reduction of illumination, which caused an increase in the number of female plants.

Ib. Sowing of Hemp at time intervals in the open

In addition to sowing in the glasshouse hemp was sown on field plots in April, May, June, July and August of 1953 and 1954.

The experiment was repeated over two consecutive years — 1953 and 1954. The plots measured 100 × 150 cm. with twofold repetition.

The sexual index and period from sowing to flowering was followed for male and female plants.

Further the size of the plants in correlation to the period required before flowering was followed.

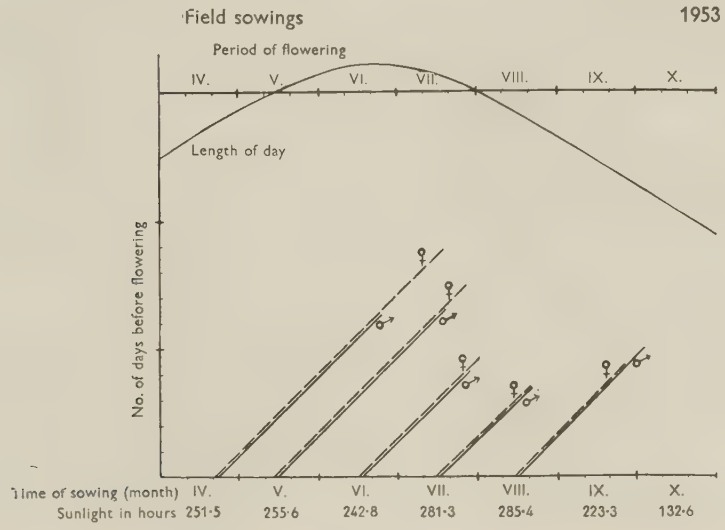
The height of the plants decreased with the shortening of the time needed for flowering: in 1953 the average height of plants from the first sowing was 175 cm., from the last sowing 9 cm.; in 1954, 190 cm. and 35 cm. The experimental results are set out in tables and graphs 3 and 4.

With regard to the period required from sowing to flowering, in 1953 the difference between male and female plants of the April sowing was very considerable; for male plants the time required was 15 days shorter than for female plants. This difference was less for later sowings: for the May sowing by 9 days, for the June sowing by 5 days. For the July sowing the period required from sowing to flowering is the same for male and female plants and for the August sowing it is 7 days longer in male plants than in female.

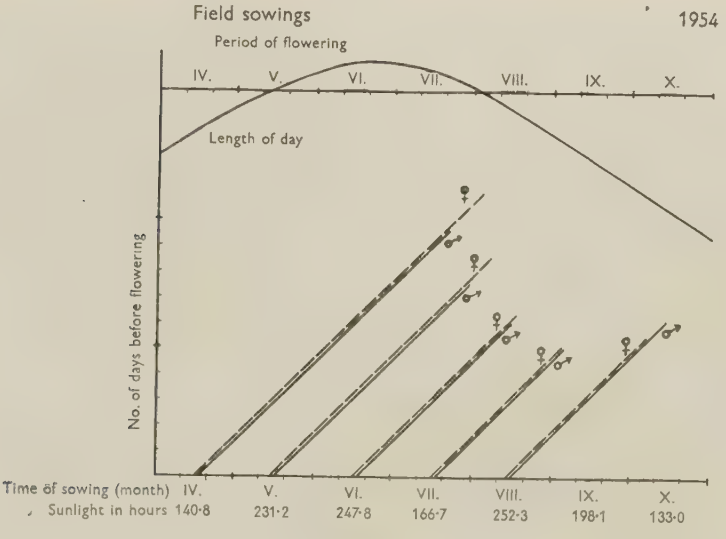
In 1954 the time required before flowering was 14 days shorter for male plants of the April sowing than for female, for those of the May sowing 10 days and for the June sowing 3 days. For the July sowing this period was the same for male and female plants and for the August sowing it was 3 days longer for male plants than for female.

There were differences between results for the two years, but they were

quantitative and not qualitative. The general rule to be deduced from experiments in the open seems to be the same as that deduced from the glasshouse experiments: female plants are short-day (i. e. shortening day) photoperiodic plants, male plants are neutral with a tendency to be long-day plants.



Graph 3.



Graph 4.

A further experiment was carried out in order to demonstrate that sex is unstable and influenced by photoperiodism.

Hemp was sown in the glasshouse on November 18 and left to grow until

Table 1

1953

Date of sowing	Number ♀ plants	%	Start of flowering		Number of days before flowering	
			♂ plants	♀ plants	♂ plants	♀ plants
21. 4.	276	57·5	23. 6.	18. 7.	64	89
14. 5.	15	60·0	18. 7.	27. 7.	66	75
15. 6.	301	58·3	27. 7.	1. 8.	42	47
15. 7.	125	54·3	20. 8.	20. 8.	35	35
14. 8.	25	50·0	3. 10.	26. 9.	51	44

Table 2

1954

Date of sowing	Number ♀ plants	%	Start of flowering		Number of days before flowering	
			♂ plants	♀ plants	♂ plants	♀ plants
16. 4.	148	58·3	21. 7.	4. 8.	97	111
15. 5.	140	50·5	29. 7.	7. 8.	76	86
15. 6.	170	55·6	14. 8.	17. 8.	61	64
15. 7.	385	51·8	3. 9.	3. 9.	51	51
13. 8.	99	48·5	13. 10.	6. 9.	61	54

it flowered. The plants flowered on March 10 and were intersexual. On March 15 they were decapitated above the third node. They sent out new shoots, which flowered again on April 16 and were again intersexual. Decapitation was repeated. The plants again came into flower and flowered from May 25 as male plants—in the period of longer days.

II. Pilot experiments were carried out on the effect of the artificial reduction of illumination on the sexual index of hemp plants.

Illumination has a certain effect on the formation of the male or female sex in dioecious plants both of hemp (Borthwick and Scully 1954, Heslop-Harrison 1957) and of hop (Valášková 1952). This is also supported by the second series of the present author's experiments. An attempt was made to ascertain the phase of ontogenesis at which this effect is exercised. Hemp was sown on plots measuring 1·5 m². Reed screens fixed in pairs on 150 cm.-high constructions were used to reduce light intensity. These screens were set up for varying numbers of days at varying periods following the sprouting of the plants. Control plots were not screened. In 1953 the hemp was sown on May 22, in 1954 on May 12, and nearly all plants had come up within a week. Plants shaded for 21 days were partially etiolated after shading and were shorter than those on the control plot, later, however, they nearly equalled the control plants in growth.

Fig. 1.

Fig. 2.

Fig. 1 and 2. Intersexual flowers in hemp.

In both 1953 and 1954 screening was carried out: a) immediately after the plants came up, b) 7 days after coming up, c) 14 days after coming up—in every case for 7, 14 and 21 days.

The increase in the proportion of female plants obtained by shading was, as compared with the unshaded control, in 1953 5·1—6·1%, in 1954 1·6 to 4·5%, average for both years 4·32%. Differences in these pilot experiments between the variants were small and probably insignificant.

Nevertheless, concurrent results for the two years show that changes in the sexual index caused by decreased light intensity depend on the ontogenetical age of plants and the duration of the decrease in light intensity. The most effective was shading which started immediately after the plants came up, or that starting a week after coming up and lasting 14 to 21 days (1954 results), or shading starting 7 or 14 days after the plants came up and lasting 14 to 21 days (1953 results).

According to these preliminary experiments it would seem that some kind of preformation of sexual polarity takes place at least partially in the period up to 14 days after the plants come up if the experimental conditions last for 14 to 21 days. In this case it would be necessary to determine the time-limits for the influencing of sex formation in the periods mentioned. These experiments will be continued.

III. The development of intersexes in hemp

In scarcely any other plant species do intersexual plants occur so plentifully and regularly—only under certain environmental conditions, however—as they do in hemp. In their habit the plants are male, but with the difference that in addition to male sex organs, stamens, they also bear female organs, pistils and ovaries with stunted seeds. This is the case only in some flowers (photo).

The cause of the development of intersexual plants and the time of their occurrence had not been sufficiently elucidated. On uncovered plots in our latitudes and climatic conditions they had not been observed by the author nor recorded in the literature.

In the glasshouse experiments already described (v. I) intersexual plants were found in 1952—1954 only from autumn to spring, or in individual cases among plants sown in September to October, and then abundantly among plants sown from November to February (22% to 30%). Among plants sown in March they were not to be found.

It was noticed that the smallest number of male plants occurred among those sown in November, December and January, i. e. when the days were at their shortest (10—8 hours). With the decrease in male plants their place was partially taken by intersexual plants. Even if the intersexual plants were added to the number of male plants, the decrease in the proportion of male plants would be greatest for the December sowing. In the January sowing their proportion would exceed that of the female plants and in the March sowing the proportion of male plants would go down to normal. Intersexual plants did not occur in later sowing. In the September sowing intersexual

plants again began to appear and they flowered after the same period from the date of sowing as the female plants.

The time required by intersexual plants to come to flower varied according to the month of sowing. Intersexual plants sown in December required 10 days less before flowering than the female plants. In later sowings they flowered at the same time as the female flowers. Male plants, however, sown in January and February required in all cases a longer period than female and intersexual plants. Intersexual plants of the November sowing started to flower on March 10. Those of the February sowing started to flower at the latest in April 9. Thus new intersexual plants did not appear once the day length had reached $13^1/_2$ hours. Experiments with reduced light intensity, which showed that this influence is effective on young plants, also lead to the conclusion that here, too, the influence of the short days in November to February, when the plants were still young, had a decisive effect on the formation of intersexes. Of all the methods of changing sex which have been tested on hemp, photoperiodism has been the only one to prove successful (Breslavec 1937).

On the basis of his experiments Schaffner (1931) came to the conclusion that the fluctuation of sex in male hemp plants is primarily caused by photoperiodic influences.

In the light of the present experiments it can be assumed that intersexual plants are actually male in the first place and that the influence of the short day has made a pure sexual differentiation impossible in them. It is further considered that the occurrence of these plants is limited to the shortest-day period, although in view of the presence of both sexual components they should represent some kind of mean between plants requiring long and short days for flowering, i. e. between male and female plants. In the opinion of the author intersexual plants are plants which have been diverted from the normal development of sexual polarity by their environment (very short days with low light intensity).

This instability and the very existence of intersexual plants are, in the opinion of the author, a clear indication of the fact that the elements of both sexes are present in the hemp seed and that one of them is more or less suppressed during development.

IV. Grafting of plants of opposite sex

With a view to the possibility of changing sex in hemp, plants of opposite sex were grafted on to each other. Grafting was carried out at a stage of growth in which the sex of the plant was usually recognizable. Males were grafted on to female stocks (10 cases), and females on to male stocks (10 cases). No change of sex occurred in any of these either in the graft or the stock.

Similar results are given by Kuhn (1941). He found that grafting had no effect on sex and no such change could take place owing to the short period of approximation. Undoubtedly photoperiodic influences also played their part, which was unknown to Kuhn—female plants do not flower when the days are long. In his sowings only male plants flowered during the long days.

He states that he knows of no cases in which grafting has caused a sex change. Limberk (1954) showed that in the European hop a sex change can take place, although only to a slight extent, following a long symbiosis of the graft and stock when a special method of grafting was used (a root onto young wood). The change occurred in buds growing on the root.

It seems most likely that a sex change in hemp could be brought about by grafting at a very early stage of the plant's development, before complete sexual differentiation has taken place. So far, however, no reliable method of determining this stage is known.

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Влияние фотопериода на половой индекс конопли

ЯРОСЛАВ ЛИМБЕРК

Резюме

От ноября до августа нами поочередно высевалась конопля (*Cannabis sativa* L.) и, сравнивая разные сроки посева, мы следили за периодом цветения мужских и женских растений. Мужские растения цветут обычно раньше женских. Если в апреле длительность освещения выше 14 часов, женские растения перестанут цвести и только продолжают расти. К цветению переходят при сокращении дня в августе, когда длина дня около 15 часов. Мужские растения цветут и в условиях более чем 14-часового освещения. В опытах по затенению растений нами установлено, что снижением интенсивности света повышается число женских растений. Нами установлены интерсексуальные растения в условиях короткого дня (11—13 часов) в количестве 22—30%. При удлинении дня на больше, чем 13 часов, развития интерсексуальных растений не наблюдается. Прививками растений разного пола — вероятно из-за короткого вегетативного сближения — не достигнуто никаких изменений пола.

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Electron Microscopy of the Aster Yellows Virus (*Leptomotropus callistephi* Ryzhkov)

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Souhrn

V homogenátech částí rostlin *Cirsium arvense* Scop., *Cineraria hybrida* hort., *Reseda odorata* L., *Taraxum officinale* Web., *Gladiolus imbricatus* L. nakažených žloutenkou aster a v *Cuscuta europea* L., která přenesla virus žloutenky aster z *Cirsium arvense* na *Petunia hybrida*, byly zjištěny stejné sférické (mnohohranné) částice velikosti 30—50 milimikronů, nejčastěji 35—40 milimikronů, které mohou být na základě srovnání s vyšetřenými homogenáty ze zdravých rostlin považovány za virospory viru žloutenky aster.

Summary

In homogenates of parts of the plants *Cirsium arvense* Scop., *Cineraria hybrida* hort., *Reseda odorata* L., *Taraxacum officinale* Web. and *Gladiolus imbricatus* L., infected by aster yellows, and in *Cuscuta europea* L., which transferred the aster yellows virus from *Cirsium arvense* to *Petunia hybrida*, similar spherical (polygonal) particles of 30—50 millimicrons, most frequently 35—40 millimicrons, were found. Comparison with homogenates from healthy plants showed that these may be considered to be virospores of the aster yellows virus.

Introduction

The particles observed under the electron microscope in extracts of virus diseased plants are, in the view of V. L. Ryzhkov and other authors, inactive forms of viruses, i. e. virospores. They may be shaped like rods, threads,

globules or they are perfectly polygonal. The presence of virospores of different virus species in plant extracts confirms the existence of virus disease. This is particularly important in cases where the infected plant does not exhibit any symptoms of disease. In many cases the shape and size of virospores can serve as an indication in the identification of genera or species of viruses.

So far electronograms of more than 50 species of plant viruses have been published. They include electronograms of the tomato stolbur virus (*Leptomotropus lycopersici* Ryzhkov)—Vovk and Nikiforova 1955, Blattný 1958. A preliminary communication on the electron microscopy of the aster yellows virus has already been published (Protsenko 1958). The present paper presents the results of further measurements of particles found in extracts of various plant species infected with aster yellows.

Material and Methods

The following material was used: 1. *Cirsium arvense* Scop., three plants infected in the open the year before and transferred to a glasshouse. Preparations were made from etiolized shoots sprouting from the soil. 2. *Cuscuta europea* L., transferred to a diseased plant of *Cirsium arvense* (sub 1) and living on this host for 3—4 weeks. In a further experiment the virus was transferred by means of this dodder on to *Petunia hybrida* hort. Pieces of stem, 1·5 to 2 cm. long, were used for the preparations. 3. *Cineraria hybrida* hort. infected in the wild state. Pieces of axils and leaves were examined. 4. *Reseda odorata* L. infected by artificial grafting (fig. 1). Pieces of shoots from one plant were used for the preparations. 5. *Taraxacum officinale* Web. infected in the wild state; leaf blades and leaf veins of two plants were examined. 6. *Gladiolus imbricatus* L., infected with "grassy top" disease (fig. 2). According to Smith and Brierly (1953), this disease is caused by aster yellows virus. The corresponding parts of healthy plants were used as controls.

Pieces of stems and lateral shoots 1—2 cm. in length, or pieces of leaves 0·5 cm.^{2}, were used for the preparations. The material was homogenized in a mortar and 5—10 ml. of distilled water was then added. Drops of the diluted homogenate were transferred with a platinum loop on to the membrane on the net. The preparations were shadowed with chromium. The sizes of the virospores were determined by measuring on the electrogram with magnification of 4600 × 4·4. A Soviet made electron microscope, EM3 1953, was used.

Results and Discussion

In a number of cases a considerable number of separate, small, spherical particles were observed on electronograms of extracts of the plants mentioned which had been infected with the aster yellows virus. The author's opinion that these particles are virospores is based on the following points: 1. In extracts of plants lacking symptoms of infection such particles were not observed in any significant numbers. 2. Identical particles were observed in extracts of different plants infected with the same virus. 3. Identical particles as in *Cirsium arvense* and other plants were found in dodder (*Cuscuta europea*) which was used to transfer the aster yellows virus. 4. Similar particles were found in extracts of tomato plants and convolvulus, infected with a related virus namely stolbur (Vovk and Nikiforova 1955, Blattný 1958)

Particles from an extract of *Cuscuta europea*, which had been living as a parasite on *Cirsium arvense* infected with aster yellows are shown on the electronogram (fig. 3). The number of particles in preparations from different plants and their different organs fluctuated considerably. Measurements of

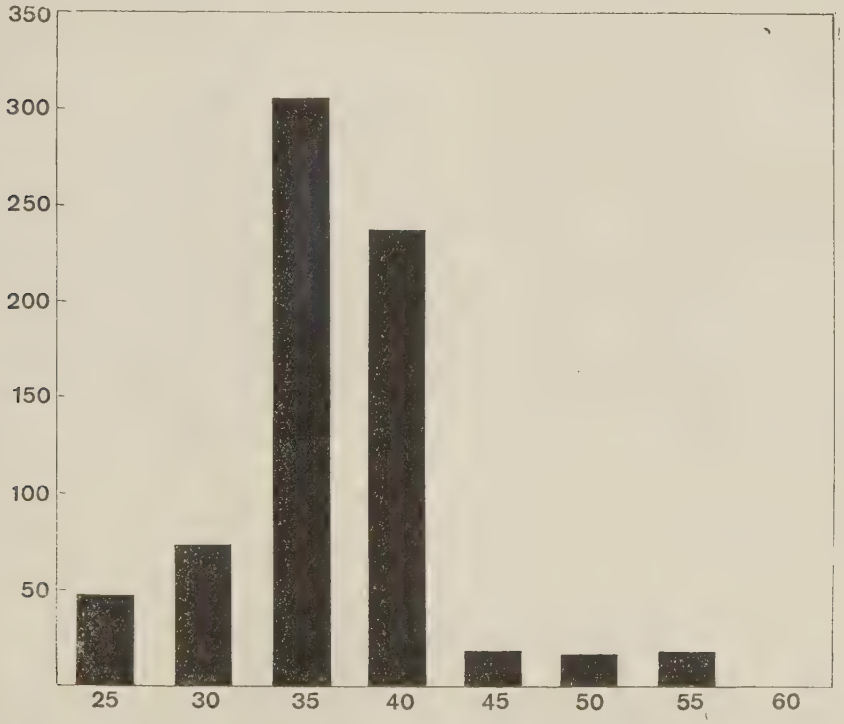

Fig. 4. Graph of sizes of particles, considered to be virospores, from various species of plants infected with aster yellows virus. Ordinate in mμ, abscissa-number of cases.

the particles showed that they average was 35—40 millimicrons. The actual size is obviously smaller. The error in these measurements is due to the limited capacity of the electron microscope as regards differentiation.

It is not as yet possible to verify whether these particles are actually virospores of the aster yellows virus or simply an artefact, because no method of separating them from various cell elements and then demonstrating their infectivity is yet known. Nor it is possible in this case to use the electron microscope in order to compare particles in a purified extract with those in the raw extract, because the aster yellows virus has not yet been purified. Thus this control is impossible at present both for aster yellows and stolbur.

It has not been possible to identify these particles on the electronogram of extracts of various organs of infected plants in all cases. It may be that plant sap decomposes them, or that the virus is inactivated by atmospheric oxygen so that the shapes of the virospores are changed: disturbing influences of

Distribution of particle sizes in extracts of plants infected with aster yellows virus

Species	Millimicrons								Total
	25	30	35	40	45	50	55	60	
Cirsium arvense	—	—	104	30	4	—	11	—	149
Cuscuta europea	—	—	30	97	13	7	3	—	150
Cineraria hybrida	—	—	4	89	3	4	3	1	104
Gladiolus imbricatus	—	—	88	12	—	—	—	—	100
Taraxacum officinale	48	—	69	1	—	6	1	—	125
Reseda odorata	—	75	11	9	—	—	1	—	96
Total	48	75	306	238	20	17	19	1	724

other factors can be assumed to exist. The concentration of virospores must also be high enough for them to be identifiable among the shapeless particles of various sizes which are produced from the disintegrated cell elements. It must also be borne in mind that virospore concentration fluctuates considerably with the age of the plant, temperature of the environment and other factors.

Acknowledgement:

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Fig. 1. *Reseda odorata* L. infected with aster yellows virus.

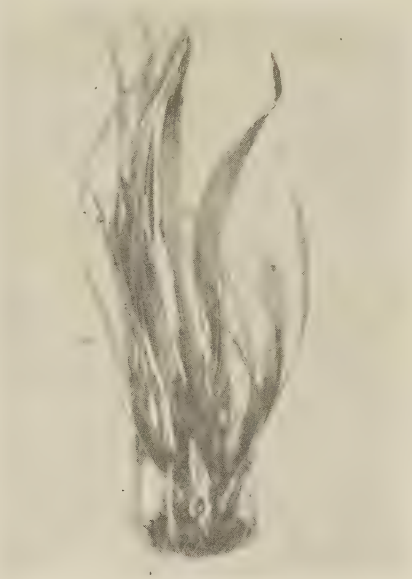

Fig. 2. *Gladiolus imbricatus* L. infected with aster yellows virus.

Fig. 3. Electronogram of an extract of *Cuscuta europea* L. which was living parasitically on *Cirsium arvense* Scop. infected with aster yellows. × 45,000.

Электронномикроскопия вируса желтухи астр (aster yellows virus) (*Leptomotropus callistephi* Ryzhkov)

А. Е. ПРОЦЕНКО

Резюме

В гомогенатах отдельных частей растений *Cirsium arvense* Scop., *Cineraria hybrida* hort., *Reseda odorata* L., *Taraxacum officinale* Web., *Gladiolus imbricatus* L., зараженных желтухой астр, а так же в *Cuscuta europea* L., которая перенесла вирус желтухи астр с *Cirsium arvense* на *Petunia hybrida* были обнаружены одинаковые сферические (многогранные) частицы размером в 30—50 миллимикронов, которые можно считать при сравнении с исследованными гомогенатами из здоровых растений вироспорами вируса желтухи астр.

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Callose Formation in Pollen Tubes and Incompatibility

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Souhrn

Bylo sledováno množství kallosy v kompatibilních a inkompatibilních pylových láčkách kulturní odrůdy jabloně (*Pirus malus* L.) a tabáku (*Nicotiana alata* Link et Otto) po zbarvení resorcinovou modří. Poněvadž je toto barvení specifické na kallosu, je velmi vhodné k selektivnímu barvení pylových láček v čnělkovém pletivu. V inhibovaných láčkách vyrostlých po samosprášení je kallosy relativně k jejich délce nejméně dvakrát tolik než v normálně rostoucích láčkách kompatibilních. Toto je dáno u jabloně především větší délkou kallosových zátek, u tabáku vedle toho větší jejich hustotou. V láčkách kompatibilních se kallosa ukládá pravidelněji než v láčkách inkompatibilních. Intensita hromadění kallosy se v jabloňových láčkách obojího typu zvyšuje s časem. Jejich růst se naopak postupně zpomaluje. Oba tyto jevy jsou patrny především u láček inkompatibilních. Obecně tedy existuje nepřímý vztah mezi množstvím kallosy v pylových láčkách a rychlostí jejich růstu.

Byla vyslovena domněnka, že fysiologická podstata tvorby kallosy spočívá v omezené schopnosti pylových láček využívat v procesu dýchání pyranovou složku metabolisované sacharosy. Glukopyranosa může podle této představy v pylových láčkách sloužit převážně ke stavbě jejich blan, její přebytek, který vzniká zvláště v případě omezeného růstu, se v nich ukládá ve formě polyglukosidu kallosy.

Summary

1. Attention is called to the possibility of using resorcin blue for the staining of pollen tubes within the style tissue and the method of staining is described.

2. In pollen tubes, the growth of which is inhibited due to incompatibility, there is at least twice the amount of callose relatively to their length as there

is in normally growing compatible tubes. In apple this is primarily due to the greater length of the callose plugs, in tobacco also to their greater density.

3. The regularity of the formation of callose in pollen tubes increases with the rate of their growth.

4. It is suggested that the physiological basis of callose formation consists in the limited capacity of pollen tubes to use the pyran components of metabolized saccharose in the process of respiration.

Introduction

Callose occurs in pollen tubes primarily in the form of the well-known callose plugs. In addition it forms a component of pollen tube walls, where together with cellulose it composes the inner lamella (Müller-Stoll and Lerch 1957a). Callose plugs in pollen tubes are formed acropetally. During the cultivation of pollen in artificial media no particular system was found to govern their location (Müller-Stoll and Lerch 1957a, 1957b).

Following the hydrolysis of callose from the fungus *Bornetina corium* (Mangin 1910) and of that contained in the cystoliths of *Ficus elastica* and *Ficus triangularis* (Eschrich 1954) and from the sieve plates of the grape vine (Frey-Wyssling, Eprecht and Kessler 1957) D-glucose was found. As a result of a more detailed analysis of callose from the sieve plates of grape vine the presence of a polysaccharide containing chains of 1 : 3—linked β—D-glucopyranose residues was demonstrated (Aspinall and Kessler 1957).

Very little is known as yet about the physiological mechanism of the formation and function of this polysaccharide. Most frequently the conception has been advanced that the callose plugs act as mechanical barriers to the reverse flow of plasma from the growing tips to the older basal parts (Molisch 1923, Eschrich 1956). Müller-Stoll and Lerch (1957b) put forward a number of objections to this conception and express the opinion that "callose is a necessary product of metabolism in pollen tubes, probably originating after a special irritation in the cytoplasm in contact with the cell wall and which may be locally accumulated under conditions of degeneration". Linskens and Esser (1957) pointed out the connection between callose formation in pollen tubes and incompatibility. After staining with fluorochrome they observed the number and size of callose plugs in the styles of autosterile petunias. They ascertained that in incompatible pollen tubes their number was nearly twice as great as in compatible tubes. The size of the callose plugs was the same in both cases.

The aim of the present authors' work has been to study callose formation in relation to the growth rate of pollen tubes in styles as determined by the compatibility or incompatibility of pollination. An attempt has also been made to elucidate the physiology of callose formation in the light of present knowledge. The paper further deals with the method of selective staining of pollen tubes in the style tissues.

Material and Method

Autosterile *Nicotiana alata* Link et Otto and fruiting apple trees (*Pirus malus* L.) var. Lord Lambourne on EM IX stocks were used as experimental material. In the case of apple, pollen from the variety Golden Pearmain was used for cross pollination. All experiments were carried out under field conditions. Emasculated, isolated flowers were picked at definite intervals after pollination. They were frozen freely in the air at a temperature of about —20° C and kept in the frozen state until needed. The average day temperatures during the experiments with apple were, for the first day 17·5° C and for the second day 21° C. The experiments with tobacco took place at average day temperatures of 14° C, 14·2° C and 11° C.

For the purpose of following the formation of callose plugs in pollen tubes grown in artificial media, pollen was cultivated in a 10% solution of saccharose containing 0·001% H_3BO_3 at a temperature of 25° C. The concentration of pollen was 5 mg. in 0·5 ml. of culture solution over an area of 10 cm².

Results were worked up by usual statistical methods. In the case of the length of callose plugs n = 120, in determining the distances between them 30 measurements were made in each case.

Results and Discussion

1. Method of staining pollen tubes

One of the specific reagents for callose is resorcin blue prepared according to Eschrich (1953). It was found that this stain is also admirably suited to the staining of pollen tubes in the style tissue. Only callose in the pollen tubes is stained blue, while the style tissue remains unstained. In view of the fact that resorcin blue does not stain callose following fixation and conservation by the usual methods which involve dehydration of the object, the styles must be treated in the fresh state or else they can be frozen and kept for any length of time until they can be treated. The advantage of this method is that the preparation of the conducting style tissue is easier after thawing. The tubes stain very rapidly and the preparations can be examined immediately. When more pronounced staining is required it is necessary to leave the stained specimens for at least a day in a moist environment. The chief points in favour of this method of staining pollen tubes are its specificity and simplicity.

2. The accumulation of callose in compatible and incompatible pollen tubes

Results of the measurement of size of callose plugs and their distances from each other are set out in tab. 1 and 2.

In the case of apple striking differences can be seen here between the amount of callose in pollen tubes from self-pollination and cross-pollination and its progressive accumulation in respect to the growth rate of the tubes. The differences in distances between callose plugs are not significant, but 24 hours after pollination their length in the short incompatible pollen tubes is nearly twice the length of those in the longer compatible pollen tubes (fig. 1 and 2).

Tab. 1. Length of and distance between callose plugs in apple

	Number of hours after pollination	Approx. length of pollen tubes	Length of callose plugs	Distance between callose plugs
		μ	μ	μ
Self-pollination	24	4400	13·83 ± 3 . 0·62	428 ± 3 . 15·65
	48	4900	Accumulation of callose along almost entire length of pollen tubes	
Cross-pollination	24	5600	7·44 ± 3 . 0·18	462 ± 3 . 15·72
	48	11000 = style length	21·70 ± 3 . 0·81	490 ± 3 . 17·79

Following the second day, when the incompatible tubes had grown through a very short section of the style, they were stained by resorcin blue for almost their entire length (fig. 3). The amount of callose in the incompatible tubes is greater particularly on the second day after pollination, not only in proportion to length of the tubes, but also absolutely.

Clearly there exists here an inverse relation between the growth rate of pollen tubes and the intensity of callose formation or alternatively a direct relation between degree of inhibition of their growth due to incompatibility and the formation of this polysaccharide. The incompatibility of pollen tubes is also marked by the accumulation of callose in the swollen tips (fig. 4), a phenomenon which was never observed in the case of compatible pollen tubes.

Thus the rate of accumulation of callose in pollen tubes can serve as a physiological indication of compatibility or incompatibility in apple. Since the choice of suitable pollinators is also connected with the degree of compatibility, a basis could be provided here for working out a laboratory method for its determination.

Essentially the same results as regards the accumulation of callose in pollen tubes and its relation to incompatibility have been obtained for *Nicotiana* as for apple.

Tab. 2. Length of and distance between callose plugs in *Nicotiana* (60 hours after pollination)

	Approx. length of pollen tubes	Length of callose plugs	Distance between callose plugs
	μ	μ	μ
Self-pollination	9000	12·24 ± 3 . 0·25	562 ± 3 . 56·80
Cross-pollination	41000	6·51 ± 3 . 0·02	1015 ± 3 . 30·10

The relatively greater amount of callose in pollen tubes from self-pollination in relation to their length is not only due to greater size but also to the greater density of the callose plugs. There is an evident regularity in the spacing of callose plugs in compatible pollen tubes in comparison with that in incompatible tubes. As in the case of apple (v. tab. 1). the length of callose plugs in *Nicotiana* is more regular in tubes formed after cross-pollination than in those with inhibited growth. In addition, in the case of incompatible pollen tubes an abnormally increased accumulation of callose in the cell wall sometimes occurs, in others sections of some mm. in length are found to be uniformly stained with resorcin blue. Callose is very frequently accumulated to varying degrees in the expanded tips of this type of tube (fig. 5 and 6). In compatible tubes, on the other hand, callose is laid down exclusively in the form of short, regular plugs (fig. 7) and is never formed in the tips of the tubes (fig. 8). The distance of the first callose plug from the tip of the tube is here about 6 to 7 mm.

The way in which callose collects in incompatible pollen tubes is very similar to the irregular formation of callose described for pollen tubes grown in an artificial medium (Müller-Stoll and Lerch 1957a, 1957b). In this connection a comparison was made for apple between the regularity of plug formation in tubes growing in the style and those growing in an artificial medium. While in natural conditions the median error of the average for values expressing the distance between callose plugs is approximately 16. the median error for the corresponding values for tubes grown in vitro is 29·54. It was further ascertained that a greater amount of callose is formed in apple pollen tubes in artificial culture than under natural conditions of growth in the style (v. seq.). In the light of these facts it can be assumed that the increased accumulation of callose in incompatible pollen tubes is not a primary result of incompatibility, but that it is caused secondarily by inhibition of their growth. In general it can be said that the more optimal the conditions for pollen tube growth the more regular its formation and the smaller its amount.

3. An attempt to elucidate the physiology of callose formation in pollen tubes

It is well known that the best medium for the cultivation of most pollen in vitro is one containing saccharose. This fact could be explained by Lvov's hypothesis (1950) on the significance of saccharose as an active carbohydrate for respiration. It is supported by the observation that of three sugars used, glucose, fructose and saccharose, germinating pollen takes the greatest amount of saccharose from the environment for its respiration (O'Kelley 1955). Similarly, in styles through which pollen tubes have grown primarily a decrease in saccharose can be observed (Linskens 1955). According to Lvov (1950) saccharose is of importance as a substrate for respiration mainly in older organs, where the enzymatic systems which activate hexoses and make them available for the respiratory process, are ceasing to act. He further assumes that the significance of saccharose consists in its active fructofuranose content and that respiration in older organs takes place exclusively

at the expense of this active furan component. Glucopyranose then remains as a secondary, unconsumed component of the respiratory process. If it is assumed that there exists a weakly active enzymatic system for the utilization of pyran forms of sugars in pollen tubes, this would serve as an explanation of the formation of the polyglucoside callose in them, the function of which is as yet unknown. According to this hypothesis the glucose part of metabolized saccharose in pollen tubes is mainly used as a component in the formation of their walls, while the residue, as a result of the limitations of its use in respiration, is laid down in the form of polysaccharide callose.

In this manner it is possible to explain the increased accumulation of callose in pollen tubes which are inhibited by the incompatibility reaction. Where growth is inhibited the use of glucopyranose for the formation of the tube walls is not possible to such an extent as in rapidly growing pollen tubes. If at the same time sugar consumption in incompatible pollen tubes is approximately the same as in compatible ones (Linskens 1955), much more unconsumed glucose will remain in the short inhibited tubes, particularly in proportion to their length, with the result that callose will accumulate to a greater extent. Similarly, in artificial medium, where optimal conditions for the growth of pollen tube are not present, callose accumulates more than under natural conditions of compatible pollination. Thus for example, in the case of pollen from the apple variety Golden Pearmain the length of callose plugs in cultures in vitro was 33·37 $\mu \pm 3 . 3{\cdot}19$ after 24 hours, the distance between them was 241 $\mu \pm 3 . 29{\cdot}54$; in pollen tubes of the same variety growing in styles these values the day after pollination were, as has already been stated, 7·44 $\mu \pm 3 . 0{\cdot}18$ and 462 $\mu \pm 3 . 15{\cdot}72$.

In the same way it is possible to explain the fact that the amount of callose in pollen tubes increases with the concentration of saccharose in the nutrient environment not only absolutely but also in proportion to length (Müller-Stoll and Lerch 1957a); where there is a sufficiency of saccharose in the environment a pollen tube consumes the easily metabolized furan component primarily in respiration, while pyranose is deposited to an increased extent as its polymer callose.

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Образование каллозы в пыльцевых трубках и явление несовместимости

ЯРОСЛАВ ТУПЫ

Резюме

Мы изучали количество каллозы в совместимых и несовместимых трубках пыльцы культурных сортов яблони (*Pirus malus* L.) и табака (*Nicotiana alata* Link et Otto) после окраски их резорциновой синей. Так как окраска является специфической по отношению к каллозе, то она весьма удобна для селективного окрашивания пыльцевых трубок в тканях пестика. В ингибированных трубках, развившихся после самоопыления, количество каллозы по отношению к их длине не менее, чем в два раза завышено по сравнению с нормально растущими совместимыми трубками. Это явление у яблони вызвано, прежде всего, большей длиной каллозных затычек, у табака, кроме того, еще и большей их густотой. В совместимых трубках каллоза откладывается более регулярно, чем в несовместимых. Интенсивность накопления каллозы обоих типов пыльцевых трубок яблони повышается со временем. Наоборот, рост их постепенно замедляется. Оба эти явления заметны, прежде всего, у несовместимых трубок. Следовательно, общим явлением является косвенное отношение между количеством каллозы в пыльцевых трубках и скоростью их роста.

Высказано предположение, что физиологическая сущность образования каллозы заключается в ограниченной способности пыльцевых трубок использовать в процессе дыхания пирановую часть метаболизированной сахарозы. Глюкопираноза, следуя этому представлению, служит в пыльцевых трубках преимущественно для постройки их стенок. Ее избыток, возникающий особенно в случае ограниченного роста, откладывается в форме полиглюкозида каллозы.

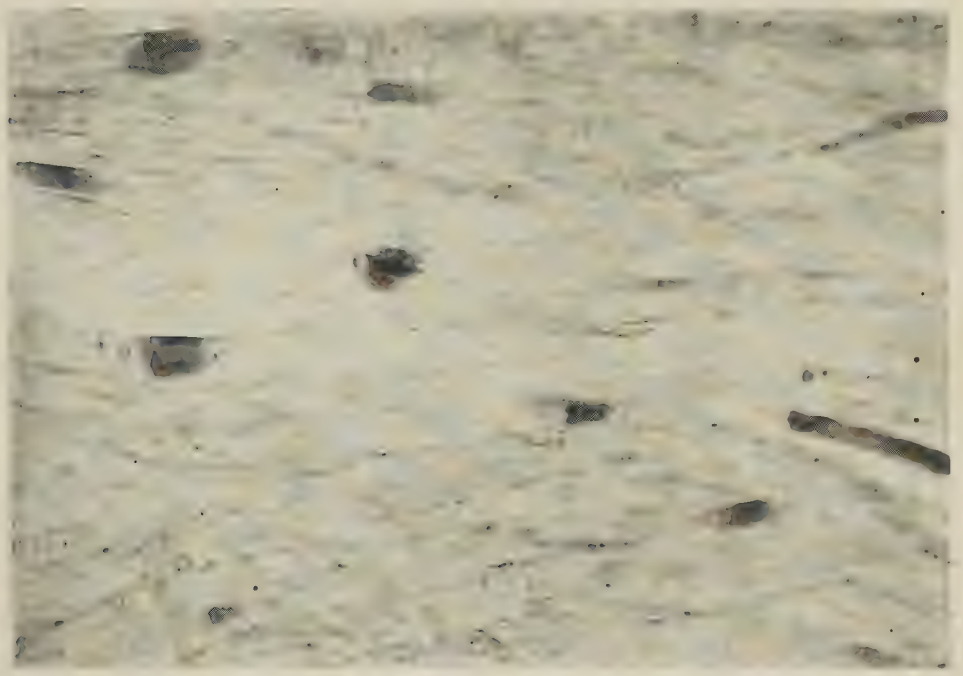

Fig. 1. Callose plugs in incompatible apple pollen tubes 24 hours after pollination (magn. × 575).

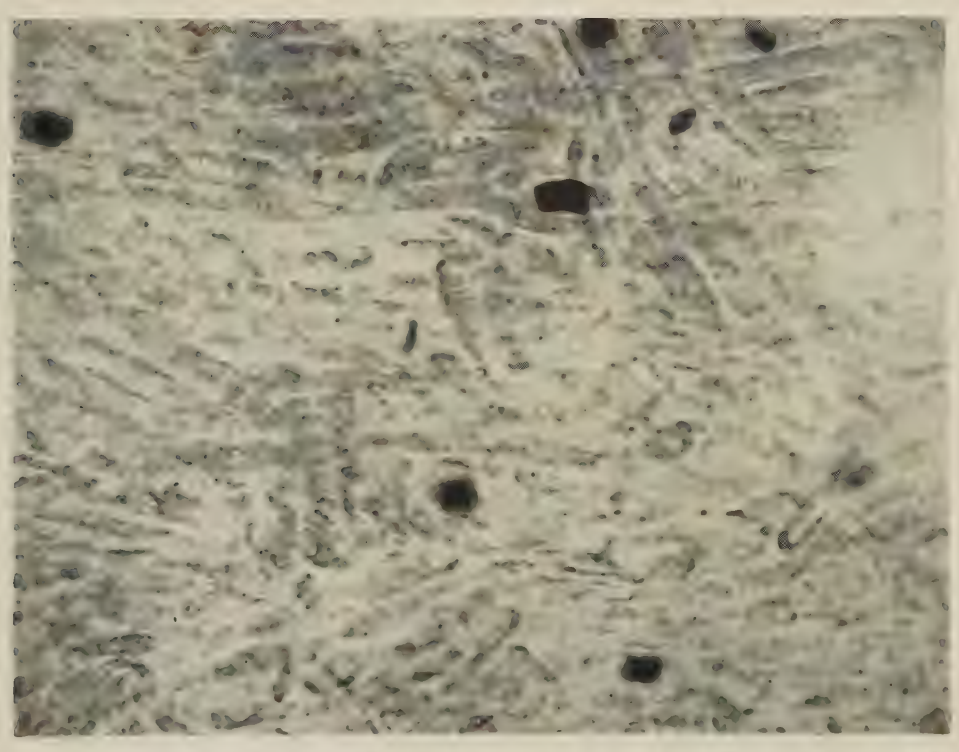

Fig. 2. Callose plugs in compatible apple pollen tubes 24 hours after pollination (magn. × 575).

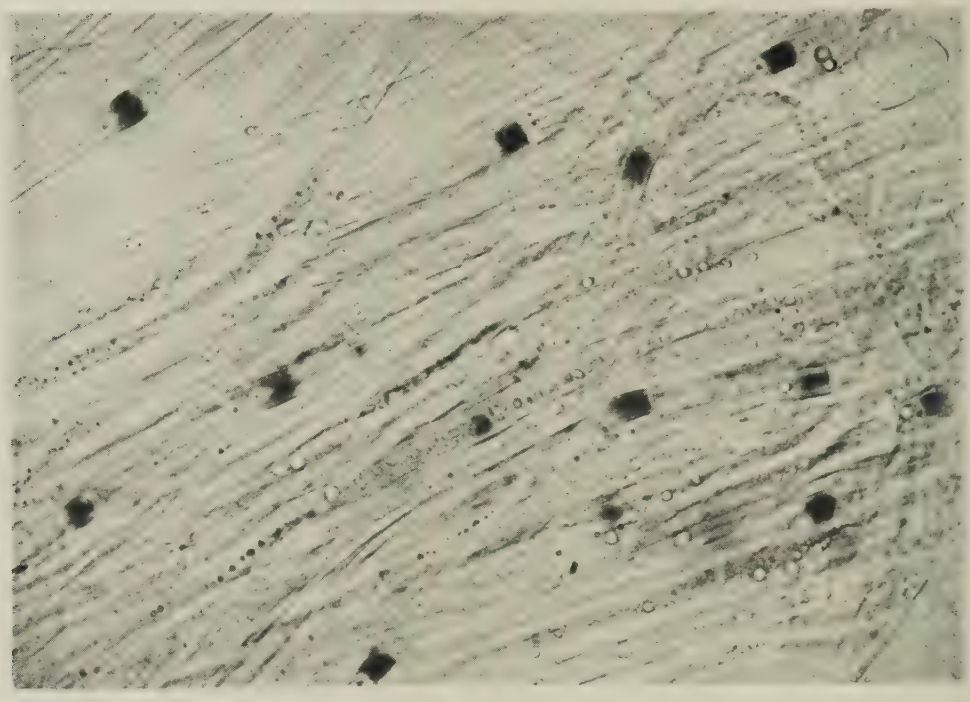

Fig. 7. Callose plugs in compatible *Nicotiana* pollen tubes (magn. × 575).

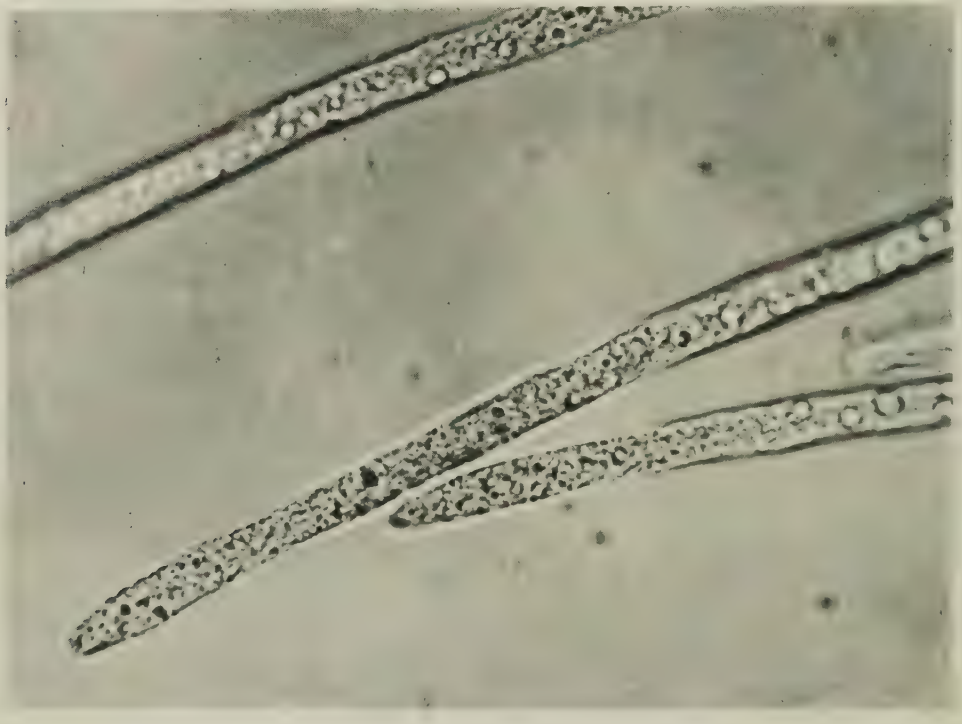

Fig. 8. Tips of compatible *Nicotiana* pollen tubes (magn. × 1150). Photo: J. Kubec

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The Application of Extracted Humus Substances to Overground Parts of Plants

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Souhrn

V předložené práci byl sledován vliv jednotlivých isolovaných látek humusu při mimokořenové aplikaci postřikem na list. Studované složky humusu ukázaly rozdílný vliv na růst, procesy dýchání i na obsah chlorofylu u rostliny *Begonia semperflorens* Link et Otto. Výsledek fysiologických změn se obráží i v morfologických změnách nadzemní části a zejména ve stavbě kořenového systému. Fulvokyseliny se ukázaly jako nejúčinnější složka humusu při postřiku na list.

Summary

The work presented in this paper was a study of the influence of different isolated humus substances when applied to the overground parts of plants by spraying on the leaves. The humus components employed showed varying effects on growth, respiration and chlorophyll content in *Begonia semperflorens* Link et Otto. The result of the physiological changes is reflected in morphological changes of the overground parts and particularly in the structure of the root system. Fulvic acids were found to be the most effective of the humus components when sprayed on leaves.

Introduction

Work on the direct influence of humus substances on the plant is frequently published. Attention has been devoted so far to the question whether the different components of humus penetrate directly into the organs and cells, or whether their influence on the plant is indirect through increased development of root and soil microflora or the improvement of the physicochemical conditions. A new and definite approach to these questions has been revealed

for the first time by the most recent work dealing with the entry of these substances into plants, where the radioisotope C^{14} in humic acids has been used (Prát and Pospíšil 1959). The authors (Sladký and Tichý 1959) have attempted to solve this problem by the application of humus substances to the overground parts of *Begonia semperflorens*. A dialyzed extract of garden soil containing sodium humate with admixture of substances from the fulvic acid fraction was used for spraying. The humus substances were found to have a favourable effect on the growth of the plant, the respiratory process was accelerated and the chlorophyll content of the leaves increased. It was then necessary to determine which of the humus components has the strongest effect on the plant and to establish the nature of the physiological action of the different substances. As in the previous experiments, *Begonia semperflorens* growing in Sach's nutrient solution was employed as the experimental plant.

Material and Methods

Garden compost soil was subjected to fractional extraction, the humus components being progressively isolated: alcoholic extract, humic acids and fulvic acids, as in the previous experiments (Sladký 1959). Out of three hundred Begonia plants which had been cultivated up to the stage of two assimilation leaves, seventy plants of similar appearance were chosen for the experiment. The plants were rinsed with water and the roots were thoroughly cleaned of all remnants of soil. They were then placed in 200 ml. Erlenmeyer flasks containing 150 ml. of Sachs's nutrient solution with the addition of the appropriate amount of A—Z solution. The plants were fixed in the flasks by rings of cotton wool round the bases of the stems. The flasks were then placed in flower pots and covered up to their necks with sand so that the roots should be in the dark and be thermally isolated. Following a week's cultivation, four equivalent experimental series of twelve plants each were set up.

1. Control plants sprayed with distilled water.
2. Plants sprayed with alcoholic extract in a concentration of 50 mg. of extract to 1 litre of distilled water.
3. Plants sprayed with humic acids in a concentration of 100 mg. to 1 litre of distilled water.
4. Plants sprayed with fulvic acids in a concentration of 100 mg. to 1 litre of distilled water.

The concentrations used were decided by extrapolation from the experiments described in the paper by Sladký and Tichý (1959). The spraying of the overground parts of the plants was carried out with a hand sprayer twice a week for one month during the period of intense growth. The dose for one spraying of each plant was about 2 ml. For the determination of chlorophyll content of the leaves a parallel experimental series with the same number of plants was started three weeks later using plants planted out at the same time and transferred to Sach's solution in the same way. Differences between the experimental series were determined after 7 weeks of cultivation, when the following measurements were made: height and length, fresh and dry weight, water content of overground parts and of the root system converted to a unit of dry weight. Oxygen consumption of the leaves was measured with a Warburg apparatus and chlorophyll content was determined photocolorimetrically by Godnev's method (1952).

Results

The experiments showed that the different fractions of humus applied to the overground parts of *Begonia semperflorens* had varying effects on growth, physiological processes and morphological changes. The values obtained for

Table 1. Measurements and weights of *Begonia semperflorens* plants following spraying of the overground parts with isolated humus components

		Control	Alcoholic extract	Humic acids	Fulvic acids
Height of stems in cm.		6·14 ± 3 . 0·90	10·12 ± 3 . 1·49*	9·88 ± 3 . 1·36*	11·36 ± 3 . 1·47**
Length of roots in cm.		13·20 ± 3 . 0·86	15·05 ± 3 . 0·79	15·28 ± 3 . 0·74	15·02 ± 3 . 0·97
Fresh weight in g. (g_u)	Stems	7·52 ± 3 . 1·28	10·18 ± 3 . 1·63	11·39 ± 3 . 1·19*	14·52 ± 3 . 1·50**
	Roots	1·40 ± 3 . 0·20	1·58 ± 3 . 0·31	1·82 ± 3 . 0·19	2·63 ± 3 . 0·30**
Dry weight in g. (g_o)	Stems	0·36 ± 3 . 0·03	0·46 ± 3 . 0·09	0·44 ± 3 . 0·04	0·61 ± 3 . 0·09**
	Roots	0·13 ± 3 . 0·01	0·11 ± 3 . 0·01	0·13 ± 3 . 0·02	0·16 ± 3 . 0·02*
g_u/g_o	Stems	20·83	21·99	25·71	24·00
	Roots	11·20	14·23	14·19	15·84

Difference	$t_{(24)}$
* statistically significant P = 0·05	2·06
** statistically highly significant P = 0·01	2·80

growth and weight of overground parts and the root system have been dealt with statistically and their significance stated. They are given in table 1. It is evident from these results that the fulvic acids are the most active humus component when applied to leaves, for they increase the height and fresh weight of stems and roots and the dry weight of stems very significantly. The increase in root dry weight remains significant. Humic acids caused significant increases in the height and fresh weight of stems. The alcoholic extract only increased the height of stems significantly. The numerical relation between fresh weight of stems and roots and their dry weight provides an indication of the amount of water in the tissues in relation to a unit of dry weight. It was found that humus substances increased this quantity of water in the plant as a whole in all cases. Changes in oxygen intake by the leaves (determined by the Warburg apparatus and recorded in table 2) show increased oxygen consumption with fulvic acids and humic acids. The table also reveals increases in chlorophyll content in the case of leaves of the same age, particularly with fulvic acids and alcoholic extract.

The influence of the different fractions on overground parts and, in parti-

Table 2. Oxygen consumption of *Begonia semperflorens* leaves in ml. per hour converted to 1 mg. dry weight. Average of three measurements

	Control	Alcoholic extract	Humic acids	Fulvic acids
Leaves	0·980	0·978	1·274	1·387
Increase as compared with control		0%	30%	41%

cular, on the formation of the root system is characteristic, as is shown by the figures. Figures 1a to 1d give a general view of experimental plants whose apical parts remained preserved. Figures 2a to 2d show plants with the apexes injured. These develop lateral shoots from the leaf axils and the regenerative capacity of plants from the different experimental series is shown. Figures 3a to 3d illustrate differences in the formations of the root systems as a whole and figures 4a to 4d show differences in root branching from these series.

The stems of control plants, which were only sprayed with distilled water, were short with only slightly turgescent leaves and these dried up at the apex and fell off at the base (fig. 1a). Lateral shoots in the leaf axils were formed in isolated cases (fig. 2a). The root system was comparatively slightly developed (fig. 3a). Lateral roots were mainly formed on the upper half of the root, were fairly short and little branched (fig. 4a).

Plants sprayed with alcoholic extract formed longer stems with fully turgescent leaves of fresh green colour and less prone to fall (fig. 1b). The apical parts of some plants dried up, but by regeneration light-green stems developed in the leaf axils (fig. 2b). The root system was more developed (fig. 3b), lateral roots were long and branched (fig. 4b). Spraying with humic acids gave rise to stems with fully turgescent leaves (fig. 1c). Adventitious shoots were formed in the leaf axils of some plants with dried apexes (fig. 2c). The effect of spraying with humic acids was most evident in the formation of a richly developed, fine root system (fig. 3c) with long and much-branched lateral roots (fig. 4c). Following the application of fulvic acids to the leaves the longest stems were formed, the leaves did not fall, were fully turgescent and dark green (fig. 1d). The formation of lateral shoots in the leaf axils was the greatest in this series (fig. 2d). Fulvic acids also gave rise to a richly

Table 3. Amount of chlorophyll in 1 litre of solvent expressed in mg. per 100 mg. dry weight of *Begonia semperflorens* leaves. Average from six measurements

	Control	Alcoholic extract	Humic acids	Fulvic acids
Leaves	1·006	1·167	1·074	1·207
Increase as compared with control		16%	7%	22%

developed root system (fig. 3d) with fine and very long lateral rootlets (fig. 4d). The whole appearance of the plants indicated that the effect of fulvic acids was favourable.

Discussion

The results of this work confirm the influence of humus substances on plants when they are applied to the overground parts, and show differences in the action of the humus components. It was found that when applied to the leaves fulvic acids are a still more effective component of humus than humic acids. Spraying with fulvic acids increased the length of stems, fresh weight of stems and roots and dry weight of roots very significantly. Increased oxygen consumption by the leaves provides evidence of an intensification of synthetic processes in the light of Oparin and Gelman's work (1952). This was also undoubtedly contributed to by increased chlorophyll content of the leaves. Increase in fresh weight was partly caused by higher tissue water content. The strong root system with its characteristic formation was reflected in the general vitality of the plants. Plants sprayed with humic acids showed significant differences in greater height and fresh weight of stems as compared with the controls. Higher oxygen consumption was, in this experimental series too, reflected in increased synthesis and a concurrent rise in water content, although the chlorophyll content was only slightly raised. The root system was well developed, richly branched and fine. Alcoholic extract increased the height of stems significantly. A partial increase in chlorophyll did not result in increased synthesis, perhaps because oxygen consumption was on the same level as for the control plants. The fresh weight of the plants was, therefore, only raised by increased water content. The root system was richer, with branched lateral roots.

Insofar as it is possible to compare the results of different experimental objects, these changes in growth and metabolic processes are reminiscent of the effects of humus substances in nutrient solution, i. e. when they are available to the plant through its roots (Sladký 1959). Similarly, morphological changes in the root system show that typical changes take place as a result of overground application and that these are characteristic for the different components of humus.

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Внекорневое применение изолированных гумусовых веществ

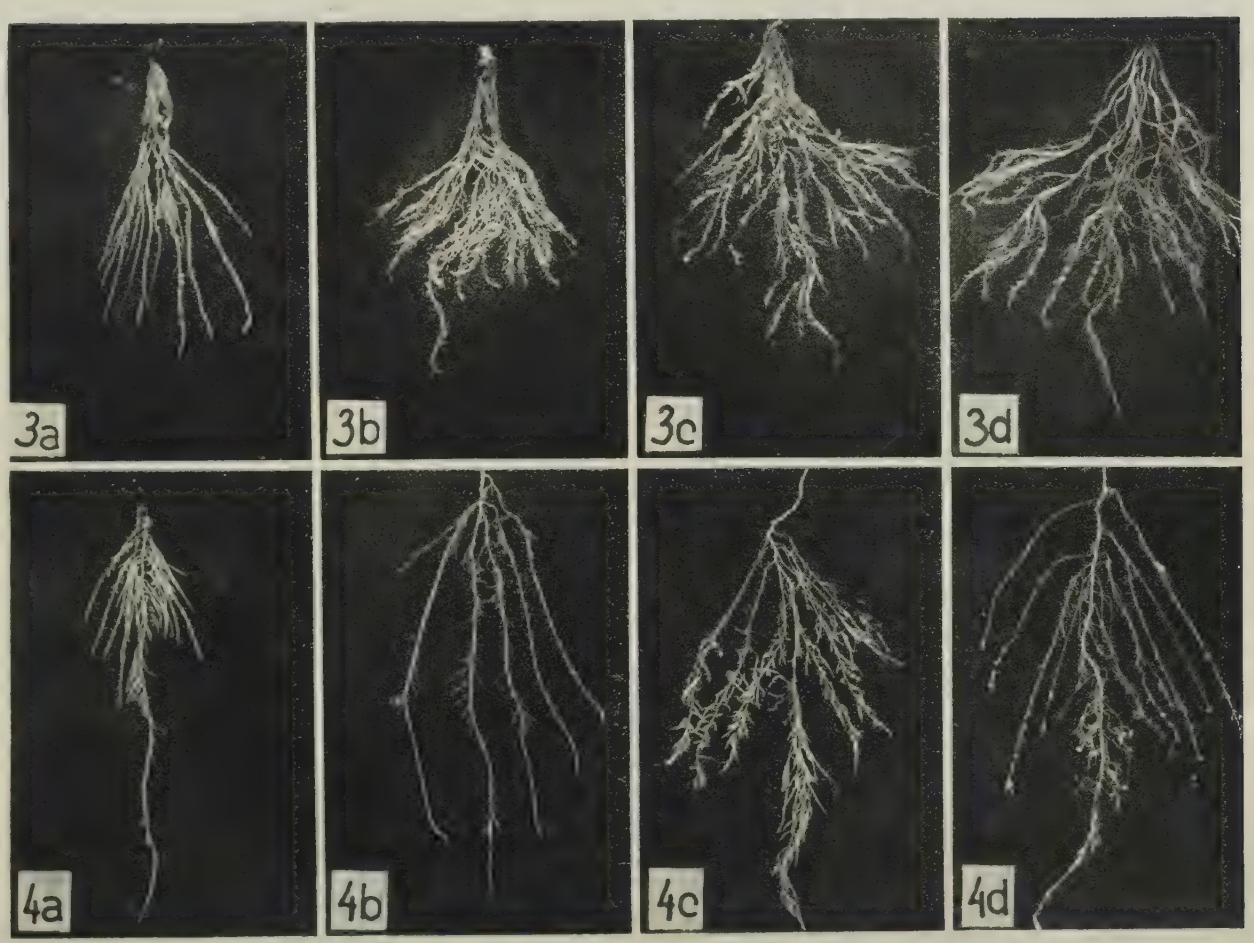
ЗДЕНЕК СЛАДКЫ

Резюме

В предлагаемой работе было прослежено влияние отдельных изолированных веществ гумуса при помощи применения их путем опрыскивания листа. Исследованные составные части гумуса показали, что они по разному влияют на рост, процессы дыхания и на содержание хлорофилла в растении *Begonia semperflorens*. В результате физиологических изменений имеют место также морфологические изменения надземной части растения и, в особенности, изменения в корневой системе. Наиболее действующей составной частью гумуса оказались фульвиновые кислоты при опытах с опрыскиванием листьев.

THE APPLICATION OF HUMUS SUBSTANCES TO OVERGROUND PARTS

THE APPLICATION OF HUMUS SUBSTANCES TO OVERGROUND PARTS



Fifty Years Since the Foundation of Rudolf Dostál's School of Experimental Morphology

Description and comparison were indispensable stages in the development of morphological methods. They were, however, incapable of establishing the real factors governing the formation of the plant organism. The discovary and ultimate control of these factors can only be achieved through scientifically directed experiment. The realization of this fact gave rise to experimental plant morphology at the end of the last century, thanks to the work of German botanists. The task of this new branch of botany was to reveal and influence the factors governing ontogenesis and also to elucidate the mechanisms of phylogenesis. Timiryazev included the latter point among the tasks facing experimental morphology as early as 1890.

At that time no work in the field of experimental morphology was being carried out in Bohemia. Indeed, it was not until 1901 that Prof. Dr. Bohumil Němec, the Nestor of plant physiology in this country, founded the Institute for Plant Anatomy and Physiology as part of what was then the Philosophical Faculty of Charles University in Prague. In a comprehensive study published in 1905 Prof. Němec showed how the basic functions are correlated in plants and pointed out the resemblance between regenerative processes in animals and plants, this resemblance being due to the fact that the processes are carried out in a similar manner by certain tissues stimulated by irritation.

The foundations of experimental morphology were thereby laid and this new, modern branch of botany could be further developed.

Today, after more than fifty years, we can fully appreciate the contribution made by Prof. Dr. Rudolf Dostál, now Corresponding Member of the Czechoslovak Academy of Sciences. Czech experimental morphology could hardly have got a more able, enthusiastic and devoted worker. He took up work in this new branch of botany in Prof. Němec's institute in 1907. Dostál's attention was directed from the beginning to the fundamental aspect of experimental morphology, i. e. to the correlation between the cotyledons or

leaves and their axial buds. His papers on these correlations, published in "Rozpravy České akademie věd a umění" and in "Berichte der Deutschen botanischen Gesellschaft" in 1908 and 1909 mark the beginning of independent work on experimental morphology in Bohemia.

When we survey the fifty years that have passed since then we come to realize the extent of the contribution made by Rudolf Dostál's school to botanical science. His thorough study of correlations also played a significant part in the discovery of auxins, and he discovered and published the first descriptions of a number of forms of the practical application of these substances in agriculture. This undoubtedly represents his greatest contribution: in his profoundly theoretical discoveries he was always able to find that "ray of light for the practical workers", which sooner or later must emerge from all honest scientific work.

The totality of the plant organism is the product of opposing inhibitory and stimulatory forces. The above-mentioned first experiments by Dostál, published fifty years ago, showed that cotyledons containing rich reserves act as inhibitors. Thus, following the amputation of one of the cotyledons and severence of the epicotyl in peas, a bud always develops in the axil of the amputated cotyledon. This is undoubtedly very economical, for the plant does not divide its reserves, which have been reduced by one-half, between two weak shoots, when this correlation enables it to produce one strong shoot in compensation for the lost main stem. The effectiveness of all correlations is without question, for during phylogenesis they have progressively appeared and become established as the main means of maintaining life and the proper orientation of the plant in its environment. The regulatory function of assimilating leaves, which have a similar inhibitory effect on their axial leaves, is therefore equally effective.

Professor Dostál also attributed the periodic budding of oak and beech trees under continuous illumination to the inhibitory effect of growing leaves. He further provided experimental evidence for the connection between scale formation on the buds of woody plants and leaf inhibitions, from which the scales in fact protect the developing embryonic elements of foliage leaves or floral parts of the bud. By intensifying inhibition during the development of a new organ, either by operation or photoperiodization, or directly by the use of synthetic inhibitors, Dostál succeeded in revealing certain phylogenetic stages which are now omitted in normal ontogenesis. Examples of such phenomena are pinnation of horse-chestnut leaves, tri-partite leaves of peas, monopodial apex of lilac shoots, etc. In this way experimental morphology began to fulfil its main purpose, i. e. it began to lay the foundations for the study of evolution.

The growth of the marine alga *Caulerpa* is also regulated by the inhibitory influence of green, leaf-like assimilatory organs. Dostál discovered the reproductive organs of this alga. From the point of view of vegetative morphogenesis there is no fundamental difference between this non-cellular alga and higher plants. Its rhizoids exert a stimulatory influence in opposition to the inhibitory effect of the green assimilators in the same way as the roots of higher plants act as stimulators in opposition to the inhibition of the leaves. Apical dominance is also dependent on the interaction of these stimulatory and inhibitory influences. A detailed study of this question has been one of the latest pieces of work undertaken by Dostál. He demonstrated the stimulatory influence of roots in relation to their production of amino-acids mainly by correlation experiments on germinating flax plants.

Even before the discovery of photoperiodism, Dostál gave the first clear demonstration of the fact that the quality of the formation of organs depends

on leaf metabolism, which of course differs according to the position of the leaf on the plant and to its age. The biochemical and biophysical changes accompanying this process are still rather obscure. At the same time, according to Dostál's experiments, the apical meristems are subjected more or less passively to the influence of the other plant parts. He carried out a number of photoperiodic experiments, chiefly on members of the genera *Circaea* and *Bryophyllum*, which he included in a new group of so-called long-short-day plants, according to their photoperiodic reactions. In quite recent experiments he has further elucidated the relation of their photoperiodic reactions to gibberelin.

Dostál's study of correlation contributed to no small extent to the discovery of auxins. With his pupils he has carried out extensive work on plant morphogenesis in relation to these substances. The elaboration of methods for the practical application of the scientific discoveries of Professor Dostál's school have been entrusted to his pupils. Thanks to this work a number of scientific methods have found practical application in this country, such as artificial rooting agents, the treatment of some species of garden seeds with stimulators before sowing, the use of herbicides for destroying weeds, spraying with stimulators in gardening, the use of retardation substances to improve the keeping qualities of fruits, etc.

Professor Dostál was never content to work only in the confines of his institute. He always directed his scientific work into the wider field of assisting the development of modern crop production. His book "The Totality of Plants", which is now in the press, will provide exhaustive evidence of the value of these aspects of his work and a review of fifty years research.

J. Podešva and J. Šebánek

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Stimulation of the Growth of *Trifolium pratense* following Exposure to Low Doses of Chronic Gamma Irradiation

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Súhrn

V práci sú zaznamenané výsledky sledovania účinku nízkych dávok gama žiarenia, intenzít 1,800—0,240 r/den. Ožiarovanie bolo prevádzané na kobaltovom poli so žiaričom 0,50 g. ekv. Ra Co^{60}, počas celého vegetačného cyklu. Pokusným objektom bol *Trifolium pratense*, odrôda Jičínský. Výsledky boli sledované jednak na jednotlivých rastlinách v individuálnej výsadbe a jednak vo výseve. Výsledky z meraní a vážení rastlín boli štatisticky zhodnotené pomocou t-testu a analýzy variance. Nízke denné dávky žiarenia (1,8—0,3 /deň) vyvolali významný stimulačný účinok, ktorý sa prejavil väčšiou výškou ožiarených rastlín v porovnaní s kontrolnými a väščím výnosom zelenej hmoty. Vo výseve bolo pozorované až 40%-né zvýšenie výnosu zelenej hmoty.

V závere sú diskutované niektoré práce, ktoré boli vykonané v tomto obore. Pokusy a merania ukázali, že nízke dávky chronického gamma žiarenia môžu vyvolávať stimuláciu rastu rastlín. Práca je potvrdením výsledkov, ktoré dosiahli Breslavcová, Berezinová a Ščibrja (1956) na inom materiále.

Summary

Results of the action of low doses of gamma irradiation, intensity 1·80 to 0·24 r/day, are recorded in this paper. Irradiation was carried out on a gamma field with a 0·50 g. equiv. Ra Co^{60} source throughout the whole vegetative cycle. *Trifolium pratense* var. Jičínský was chosen as the experimental material. Growth and the yield of green matter was followed both on individually bedded out plants and on sown plots. Results of the measurement and weighing of the plants were statistically evaluated by means of the t-test and analysis of variation. Small, daily radiation doses (1·80—0·30 r per day)

produced a significant stimulatory effect which was evidenced in the greater height of the irradiated plants as compared with the controls and a higher yield of green matter. In conclusion the paper deals with work that has already been carried out in this field. Experiments and measurements have shown that low doses of chronic gamma radiation are capable of stimulating plant growth. This work is confirmed by result obtained by Breslavets, Berezina and Shchibrya (1956) on other material.

Introduction

The cultivation of plants on cobalt fields made it possible to follow the effects of radioactive radiation throughout the whole vegetative cycle. It was observed that strong doses of 50—6,000 r/day applied throughout the vegetative cycle inhibited growth and caused morphological and anatomical changes (Sparrow and Gunckel 1955, Biebel 1956) and finally genetic changes (Nybom 1956, Nybom, Gustafsson, Granhall and Ehrenberg 1956), nor does gamma radiation of low intensity applied in doses of a few r a day remain without effect. Granhall, Ehrenberg and Borrenius (1953) consider that daily doses of 0·25—0·58 r reduce fertility in barley. Sparrow and Pond (1956) found chromosome aberrations in *Lilium longiflorum* following daily doses of 0·60 r.

The stimulatory effects of low doses of gamma radiation noted by Breslavets, Berezina and Shchibrya (1956) are of a special nature. The authors found that daily doses of 0·019—0·036 r/day evoke stimulation of growth and development of maize. Similar results with buckwheat were produced by doses of 0·460—2·496 r/day.

Methods and Evaluation

The experimental plants were planted out on a field of 0·25 hectares in the middle of which a cobalt source Co^{60} of 0·50 g. equiv. Ra was placed.

The radiation apparatus was constructed as follows (fig. 1): On a steel frame (1) 150 cm. high control pulleys (3) were fixed. A cobalt source (6) was attached to the lower part of the lead stopper of the container (5) with polyacrylate. This method of attachment was found to be very satisfactory. During construction it was found that it was necessary to place a lead shade (7) above the source. The intensity of radiation without the shade, particularly behind the protective screen, was ten times greater owing to secondary reflected radiation. The control cable (2) was laid underground for a distance of 4 metres to the other side of a 30 cm. thick concrete protective wall. This radiation apparatus is portable and safe. During manipulation and field work the cobalt was passed into a protective container (4) buried underground.

One section of the field was planted with *Trifolium pratense* var. Jičínský in circles 40 cm. from each other, the plants being 25 cm. apart. The plants were one month old. At the same time the second section of the field was sown with the same variety in rows at intervals of 20 cm. During the whole period of vegetation the plants were exposed to radiation of 1·80—0·12 r/day. The control plants were placed behind the protective wall, where the radiation intensity did not exceed 0·010 r/day. In the case of the bedded out plants each plant was evaluated separately

from the isodosic points. In the case of the sown crop three areas at one metre from the isodosic points were mown and the crop from each area was weighed separately. Measurements were made when the plants were in full flower by means of t-test and analysis of variation.

Dosimetry was carried out by means of Oplt's intentdosimeter and Siemens Gamma Meter.

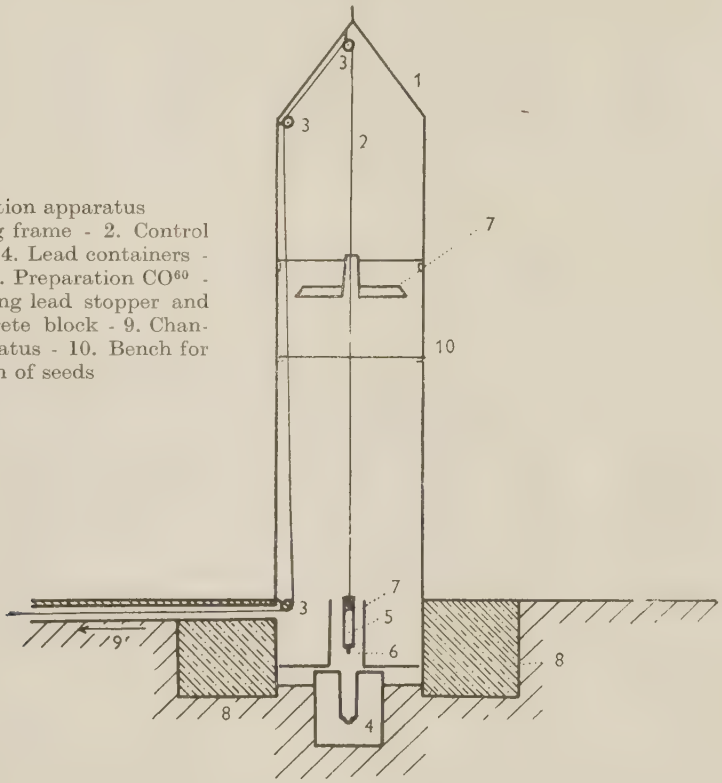

Fig. 1. Irradiation apparatus
1. Plan of supporting frame - 2. Control cable - 3. Pulleys - 4. Lead containers - 5. Lead stopper - 6. Preparation CO^{60} - 7. Apparatus for fixing lead stopper and lead shade - 8. Concrete block - 9. Channel for control apparatus - 10. Bench for irradiation of seeds

Results

Height of plants. 40—70 plants in full flower from the bedded out section were measured (see table 1).

For plants which had been irradiated with a daily dose of 1·80 r (total dose during the vegetative period 100·6 r) the average height was 5·35 cm. greater than for the controls.

This difference was statistically significant ($5 \cdot 10^{-2} > P > 2 \cdot 10^{-2}$). In the case of daily doses of 0·46 r (total dose 28·58 r) and 0·30 r (total dose 18·60 r) the average height of the plants was 8·55 and 8·60 cm. greater than for the controls. These differences are highly statistically significant. In the case of the daily dose of 0·24 r (total dose 14·88 r) the difference was not statistically significant. The results of measurements of plants receiving doses of 1·80 r per day to 0·30 r per day show that growth had been stimulated.

Weight of plants. The weight of green matter from one mowing of plants

Table 1. Height of bedded out plants in cm.

r/day	$x \pm 3 . s_{\bar{x}}$	$\lvert\bar{d}\rvert$	n	t	P
0	55·00 ± 3 . 1·65	—	47	—	—
1·800	60·35 ± 3 . 1·49	5·35	42	2·31	$5 . 10^{-2} > P > 2 . 10^{-2}$
0·460	60·55 ± 3 . 0·99	8·55	55	4·45	$1 . 10^{-5} > P > 1 . 10^{-6}$
0·300	63·60 ± 3 . 1·80	8,60	57	4·47	$1 . 10^{-6} > P > 1 . 10^{-7}$
0.240	53·60 ± 3 . 0·84	1·40	74	0·15	$9 . 10^{-1} > P > 8 . 10^{-1}$

x average
$s_{\bar{x}}$ median error of average
$\lvert\bar{d}\rvert$ absolute value of difference compared with control
n number of plants
t test of statistical significance
P probability

in full flower was determined in a similar way to the height. The results are given in table 2. Following daily doses of 1·80 r the average weight of plants increased by 19·94 g. per plant. The results are not statistically significant. Statistically significant results were obtained following daily doses of 0·46 and 0·30 r. Plants exposed to these intensities had average weights of 21·84 and 24·11 g. higher than the controls. Following these doses highly significant differences in height as compared with controls were also observed.

Table 2. Weight of green matter from one mowing of bedded out plants in g.

r/day	$x \pm 3\, s_{\bar{x}}$	$\lvert\bar{d}\rvert$	n	t	P
0	116·66 ± 3 . 6·87	—	47	—	—
1·500	136·60 ± 3 . 9·40	19·94	42	1·72	$1 . 10^{-1} > P > 5 . 10^{-2}$
0.460	138·50 ± 3 . 6·32	21·84	55	2·37	$2 . 10^{-2} > P > 1 . 10^{-2}$
0·300	140·80 ± 3 . 6·70	24·11	57	2·51	$1 . 10^{-1} > P > 1 . 10^{-3}$
0·240	103·20 ± 3 . 4·75	13·46	74	1.62	$1 . 10^{-1} > P > 5 . 10^{-2}$

Table 3. Average crop from 1 m. of sown field in g.

r/day	$\bar{x}$	P	
0	50	—	$s\bar{x} = \pm 1{,}9$
			$sd = \pm 2{,}6$
2·400	48·3	$P < 0·05$	
0·700	51·0	$P < 0·05$	
0·260	71·0	$P > 0·01$	
0·240	69·3	$P > 0·01$	
0·120	48·9	$P < 0·05$	

sd median error of difference

In order to check this experiment we evaluated the sown crop of three areas each a metre from the isodosic points. (Table 3) This sown crop was exposed to radiation for 18 days longer than the bedded out plants. Statistically significant values were obtained following daily doses of 0·26 r/day (total dose 20·80 r) and 0·24 r/day (total dose 18·40 r), in which cases the increases in weight of the mowings from metre sections were 21·0 g. and 19·3 g. respectively, that is a percentual increase in weight of 40%. These results correspond to those obtained in the case of the bedded out plants. The high ratio of variation for doses obtained by variation analysis shows that the statistically very significant differences are caused by differences in dosage.

Discussion

The question of whether ionizing radiation can act as a stimulant besides causing harmful changes has been under discussion for a long time. To all intents this discussion started following the publication of MALDINAY and THOUVENIN'S work (1898), in which they demonstrated that X-rays stimulate the germination of *Convolvulus arvensis*, *Lepidium sativum* and *Panicum miliaceum* seeds. Immediately afterwards work appeared contradicting these results (PETHERS 1923, SCHWARTZ, CZEPA and SCHINDLER 1923) and, on the other hand, work confirming the experiments (MIÈGE and COUPÈ 1914). There then followed a considerable volume of work giving varying results. A detailed survey of such work has been made by BRESLAVETS (1956).

In an attempt to use these results in agricultural practice, ZHEZHEL (1955) carried out extensive experiments involving the addition of radioactive radium and uranium salts to manures. Over a period of 8 years these substances increased crops by 20—30%. In the opinion of the author, however, this method of increasing agricultural production is extremely inadvisable, because it involves the long-term contamination of the soil with radioactive substances, which in view of the present increase in radioactivity could in time represent a grave threat. The stimulatory effects of low concentrations of the fission products of uranium were noted by TIMOFEEV-RESSOVSKY (1956). TIMOFEEV-RESSOVSKY and LUCHNIK (1958) give a biophysical description of the process of stimulation by ionizing radiation. The authors show that the basis of stimulation is the influence of low doses on accelerating cell division. They also show that the stimulatory effect of radiation is in inverse ratio to the linear density of ionization. Alpha particles do not cause stimulation at all. Of beta particles the most effective were found to be high energy particles. As regards electromagnetic quanta the short-wave rays were the most effective.

The present paper confirm the results of BRESLAVETS, BEREZINA and SHCHIBRYA (1956), pointing to the stimulatory effect of low doses of chronic gamma irradiation.

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Действие низких доз хронического гамма-облучения на рост

РОБЕРТ ГОНЧАРИВ

Резюме

В работе приведены результаты изучения действия низких доз гамма-облучения интенсивностью 1,800—0,240 р/день. Растения облучались на кобальтовом поле источником 0,50 гр. экв. Co^{60} в течение всего вегетационного периода. Подопытным объектом являлся клевер *Trifolium pratense* L. сорт Йичинский (Jičínský). Результаты измерений и взвешиваний растений статистически обрабатывались методом т-теста и анализа вариации. Низкие дозы излучения (1,8—0,3 р/день) вызвали значительный стимуляционный эффект, проявляющийся большей высотой облученных растений по сравнению с контрольными и большим урожаем зеленой массы. В посеве наблюдалось повышение зеленой массы на 40%.

В заключении обсуждаются некоторые работы, проведенные в этой области. Опыты и измерения показали, что низкие дозы хронического облучения вызывают стимуляцию роста растений. Работа является подтверждением результатов Бреставец, Березиной и Щибря (1956) на другом материале.

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Anagallis arvensis L. als Indikator der Anwesenheit von Viren aus der Gruppe der Samenlosigkeit (Akarpie) - Stolbur und Vergilbungskrankheiten

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Souhrn

Žloutenkové onemocnění *Anagallis arvensis* L. indikuje podle pozorování v přírodě přítomnost virů ze skupiny akarpií (žloutenek a stolburu). *Anagallis arvensis* L. a *Stellaria media* (L.) Vill. indikovaly v pokusech v r. 1957 přítomnost těchto chorob, též purple-topu a yellow-topu u bramborů, jestliže byli přítomni křísové *Macrosteles laevis* Rib. a *Calligypona pellucida* F. Pokus na střešní terase — při absenci obou jmenovaných druhů křísů — ukázal, že uvedené choroby nebyly přeneseny kříškem *Empoasca flavescens* F., mšicemi různých druhů, molicí *Trialeurodes vaporariorum* Westw. V roce 1958, kdy výskyt křísků byl slabší a pozdnější, indikovaly uvedené rostliny — a *Anagallis linifolia* L. — rovněž přítomnost těchto virů v bramborových porostech (purple-top a yellow-top i v podhůří) nebo v jiných reservoirových rostlinách nebo v kříscích, ale slaběji a později než v r. 1957. *Anagallis arvensis* L. a *A. linifolia* L. na některých místech indikují přesněji skupinu aster yellow (evropskou žloutenku aster), pseudoklasický stolbur, purple-top a yellow-top bramborů, na jiných skupinu klasického stolburu; příčiny toho nejsou známy. Žloutenka, přenesena křísky z *Anagallis arvensis* L. na rajčata, se u nich projevila jako pseudoklasický stolbur; yellow-top bramborů, žloutenky a klasický stolbur, přeneseny křísky na *Anagallis arvensis* L., projevily se u nich typickým zakrsáním květních korun. Silné kolísání výskytu těchto viros v přírodě (po r. 1957 s velmi silným výskytem žloutenek ve středočeských polohách následoval rok s jejich velmi slabým výskytem) je závislé na iniciálním vlivu vysokých teplot i na jiných příčinách, jako je kolísání hladiny virů v hostitelských rostlinách. *Anagallis arvensis* L., *Anagallis linifolia* L., *Stellaria media* (L.) Vill. možno použít jako indikátorů přítomnosti virů ze skupiny akarpií na určitém stanovišti. Pozorování o složení, časnosti a síle výskytu cikadofauny může sloužit k signalisaci zákroků pro nepřímý boj proti těmto virosám.

Zusammenfassung

Die gelbsuchtartige Erkrankung von *Anagallis arvensis* L. zeigt nach Beobachtungen in der Natur die Gegenwart von Viren aus der Gruppe der Akarpien (Vergilbungen) und Stolbur an. *Anagallis arvensis* L. und *Stellaria media* (L.)

Vill. zeigten bei Versuchen des Jahres 1957 die Anwesenheit dieser Krankheiten, sowie auch von purple-top und yellow-top der Kartoffeln an, sofern die Zwergzikaden *Macrosteles laevis* Rib. und *Calligypona pellucida* F. anwesend waren. Ein Versuch auf der Dachterrasse — in Abwesenheit beider genannten Zikadenarten — erwies, dass die angeführten Krankheiten weder von der Zwergzikade *Empoasca flavescens* F., noch von verschiedenen Blattlaus-Arten oder von *Trialeurodes vaporariorum* Westw. übertragen wurden. Im Jahre 1958, da das Vorkommen der Zikaden schwächer und verspätet war, indizierten die angeführten Pflanzen — und *Anagallis linifolia* L. gleichfalls — die Anwesenheit dieser Viren in den Kartoffelbeständen (purple-top und yellow-top selbst im Vorbergland), oder auch an anderen Reservoir-Pflanzen, oder in den Zikaden, aber schwächer und später, als im Jahre 1957. *Anagallis arvensis* L. und *A. linifolia* L. indizieren an manchen Standorten genauer die Gruppe der aster-yellows (der europäischen Vergilbungskrankeit der Astern), den pseudoklassischen Stolbur, purple-top und yellow-top der Kartoffeln, an anderen Orten die Gruppe des klassischen Stolbur; die Ursachen davon sind nicht bekannt. Vergilbungskrankheit, durch Zikaden von *Anagallis arvensis* L. auf Tomaten übertragen, äusserte sich an ihnen als pseudoklassischer Stolbur; yellow-top der Kartoffeln, Vergilbungskrankheiten und der klassische Stolbur, durch Zikaden auf *Anagallis arvensis* L. übertragen, äusserten sich an dieser als typische Reduktion der Blütenkronen. Das starke Schwanken im Auftreten dieser Virosen in der Natur (nach dem Jahre 1957 mit sehr starkem Auftreten der Vergilbungen in den mittelböhmischen Lagen folgte ein Jahr sehr geringen Vorkommens) ist abhängig von der Initialquelle der Ansteckung, dem Beginne des Auftretens und der Vermehrung der Zikaden, sowie auch vom inaktivierenden Einfluss hoher Temperaturen und auch von anderen Gründen, wie beispielsweise vom Schwanken des Virenspiegels in den Wirtspflanzen. *Anagallis arvensis* L., *Anagallis linifolia* L., *Stellaria media* (L.) Vill. kann man an einem bestimmten Standort als Indikatoren der Anwesenheit von Viren aus der Gruppe der Akarpien verwenden. Beobachtungen über Zusammensetzung, Termin und Stärke des Vorkommens der Zikadofaune können zur Signalisierung der Eingriffe für einen indirekten Kampf gegen diese Virosen dienen.

Einleitung

Über die Virosen, welche Blütenanomalien beim Ackergauchheil (*Anagallis arvensis* L.) im weiteren *A. a.* bezeichnet) hervorruft, siehe Blattný (1957). Im Jahre 1957 und 1958 haben wir mit dieser Pflanze weiter experimentiert. Kranke *A. a.* sowie *Stellaria media* (L.) Vill. und auch Ehrenpreisarten, vornehmlich *Veronica chamaedrys* L. oder einige von diesen Arten treten regelmässig dort auf, wo sich andere Pflanzenarten, insbesondere Kulturpflanzenarten befinden, die von Stolbur oder Vergilbungskrankheiten befallen sind. Wir nahmen darum Versuche vor, um festzustellen, ob nicht *A. a.* als Indikator dieser Virosen verwendet werden könnte. Eine vorläufige Mitteilung über diese Arbeiten siehe Blattný (1958a, b).

Dazu gesellte sich auch ein Versuch, der mit den vorhergehenden zusammenhing: Ob und zu welchem Anteil *A. a.* mit Viren aus der Gruppe der Akarpien von Kartoffeln infiziert werden kann und ob sich die Erkrankung von *A. a.* unter Terrainbedingungen auf Tomaten übertragen lässt.

Ergebnisse

I. Teil. Jahr 1957

Třebotovice bei České Budějovice. Die Versuche wurden auf einem ebenen, mit Gras bewachsenen Grundstück am 24. Mai angelegt und die Grösse der Versuchsparzelle betrug 3 × 3 m.

Versuch Nr. 1. Es wurden zehn gesunde Tomatenpflanzen gesetzt und zwischen sie (24. V. 1957 — gilt auch für die weiteren Versuche) 20 viröse *A. a.*, die im Glashaus gezüchtet und infiziert worden sind. Auf dieser Parzelle wuchsen auch noch 5 gesunde *A. a.*-Pflanzen aus Samen. Im Verlaufe der Vegetation zeigten in der Hälfte des Juni drei Tomatenstauden kleinere, anthokyanisierte Hochblätter und „gotischen" Wuchs. In der späteren Phase, Anfang August, hatten zwei dieser Tomatenpflanzen verkümmerte Blütenkronen. Die kranken *A. a.* verzweigten sich intensiv und blühten nicht. In einer Entfernung von 12 m von dieser Parzelle befand sich ein Kartoffelbestand der Sorte „Erstling", Ende April ausgesetzt.

Versuch Nr. 2. Es wurden zehn gesunde Tomatenpflanzen und zwanzig gesunde *A. a.*-Exemplare ausgesetzt. Diese Versuchsparzelle war 28 Meter von der vorhergenannten Parzelle entfernt und 40 m von den nächstwachsenden Kartoffelstauden. Bis zu Ende des Versuches blieben alle Pflanzen gesund — wurde nicht virös.

Versuch Nr. 3. Auf einer Parzelle, die von den allernächsten Kartoffelpflanzen der Sorten „Ackersegen" und „Triumf" 80 m entfernt lag, wurden zehn Kartoffelknollen mit Fadenkeimen ausgesetzt, die von Barči bei Košice stammten, wo im Vorjahre der Kartoffelbestand stark von Stolbur befallen war. In der Hälfte des Juni gaben zwei Kartoffelpflanzen mässige Anzeichen von yellow-top zu erkennen und eine Kartoffelstaude Kennzeichen, die „kudrjašče" genannt werden. Anfang August wiesen vier Kartoffelpflanzen mässige Anzeichen von yellow-top auf und dieser Stand blieb bis zur Ernte Ende September unverändert, bei welcher unter 26 Knollen, die geerntet wurden, zwei spindelförmig waren. Von diesen Knollen zeigten im Frühjahre des nächsten Jahres 22 Knollen normale Keimtriebe und vier Knollen hatten halbfadenförmige Keimtriebe.

Von den ausgesetzten *A. a.* hatten in der Junihälfte drei Pflanzen ziegelrote Blüten, die oberen Blüten hatten kleinere Kronen und zusammengedrängte Hochblätter. Anfang August wiesen bereits 8 Pflanzen typische Erkrankungsmerkmale auf (starke Reduktionen der Blütenkronen in den Gipfelpartien), was bis zum Ende der Vegetation so blieb. Ende September zeigten bei der Ernte der Kartoffeln bereits alle 20 ausgesetzten *A. a.*-Exemplare typische Erkrankungsmerkmale der Gipfelpartien. Auch von 22 Sämlingen der Art *Stellaria media* (L. Vill. zeigten drei Pflanzen und von 18 Sämlingen von *Veronica chamaedrys* L. 5 Pflanzen typische Vergilbungskrankheiten.

Versuch Nr. 4. Auf einer Parzelle, die von den nächsten Kartoffelstauden der Sorten „Ackersegen" und „Triumf" 80 Meter und von der Parzelle Nr. 3 35 Meter entfernt lag, wurden 10 Kartoffelknollen ausgesetzt, die in dieser Gemeinde geerntet worden sind, normale Keimtriebe hatten und auch von einem Grundstück stammten, auf welchem sich im Vorjahre während der Vegetation keine Stolbur-Erkrankung gezeigt hatte. Zwischen sie wurden 10 gesunde *A. a.*-Exemplare gesetzt. Bis zur Ernte blieben die Kartoffeln von diesen Knollen ohne Symptome von Virosen aus der Gruppe der Akarpien und von 10 gesunden *A. a.*-Pflanzen erkrankte nur eine Pflanze an Vergilbung.

Die Lokalität dieser Versuche ist von mittlerer Eignung für die Züchtung von Pflanzgutkartoffeln. Sorten, die gegen Virosen empfindlich sind, halten es hier ohne besondere Verbreitung dieser Virosen und ohne merkbares Absinken der Erträge 4—5 Jahre aus. In der weiteren Umgebung dieser Lokalität wurden vereinzelte Exemplare von *A. a.* und *Stellaria media* (L.) Vill. festgestellt, die an einer Vergilbung des beschriebenen Typs erkrankt waren. In den Kartoffelbeständen der Umgebung wurden vereinzelte Purple-top- und yellow-top-

Fälle festgestellt. Bei *Hypericum perforatum* L. wurde ein vereinzelter Fall von Vergilbung und bei *Trifolium repens* L. Phyllodie festgestellt. Die Population der Zwergzikaden war sehr zahlreich und es überwogen die Arten *Macrosteles laevis* Rib. und *Calligypona pellucida* F., welche die Infektionen von den kranken Versuchsexemplaren von *A. a.* und Kartoffeln in die Umgebung verschleppten, wie aus den Versuch Nr. 4. ersichtlich ist und aus einigen Fällen kranker *Veronica chamaedrys*-Exemplare in der Nähe der Versuchsparzellen 1 und 3 und aus erkrankten Kartoffelstauden der Sorte „Erstling", 12 Meter von der Versuchsparzelle Nr. 1 entfernt. Während der Vegetation äusserten diese Kartoffeln weder Anzeichen der Blattrollkrankheit, noch des pseudoklassischen Stolburs und wurden für Pflanzgutzwecke in der zweiten Augusthälfte geerntet. Proben der Knollen aus der Ernte wurden einer Vorkeimung unterzogen und von 304 Knollen zeigten zwei (d. i. 0,66%) Fadenkeimtriebe. Es handelte sich um eine Ansteckung von kranken *A. a.*-Pflanzen auf der Versuchsparzelle Nr. 1. Niemals vordem — nach Beobachtungen vom Jahre 1924 an — sind hier fadenförmige Keimtriebe beim Pflanzgut festgestellt worden. Dass es sich um eine Ansteckung im betreffenden Jahre handelte, wurde auch durch die Tatsache bestätigt, dass von den nach der Ernte als Unkraut auf dem Grundstück wachsenden Vogelmierenpflanzen [*Stellaria media* (L.) Vill.] 1,2% der Pflanzen Anzeichen von Vergilbung zeigten. Es scheint, dass Zwergzikaden der Art *C. pellucida* F. diese Ansteckung in den sonnenwarmen Tagen des Oktobers übertrugen. Sie verschleppten diese auch auf die in der Nähe angebauten Narzissen, von denen zwei von 30 Pflanzen im nächsten Jahre grüne Blüten aufwiesen, obwohl an diesem Orte Narzissen schon 30 Jahre gezüchtet worden waren, ohne dass ein Grünwerden der Blüten festgestellt worden wäre.

Die viröse Vergilbung wurde also von den kranken *A. a.*-Exemplaren auf Tomaten und von kranken Kartoffeln auf *A. a.*, *Stellaria media* (L.) Vill., *Veronica chamaedrys* L. und auch wieder auf Kartoffeln übertragen. *A. a.* indizierte also die Anwesenheit der Vergilbungskrankheit der Gipfelpartien bei den Kartoffeln, unter welchen sie wuchs.

II. Teil. Jahr 1957

Versuchsort ist der Garten der phytopathologischen Abteilung des Biologischen Institutes der Tschechosl. Akad. d. Wiss. in Praha-Dejvice.

Versuch Nr. 5. Am 20. Mai wurden Knollendrittel einer Gesamtzahl von 400 Knollen ausgesetzt, die im Vorjahre in diesem Garten geerntet worden sind. Im Jahre 1956 befanden sich in diesem Bestand etwa 5% Pflanzen mit Anzeichen von purple-top, yellow-top und auch von „Kudrjašče". Die im Bestand oder in seiner Nähe wachsenden Unkrautexemplare *A. a.* und *Stellaria media* (L.) Vill. zeigten Merkmale von Virosen, *Plantago major* L. Phyllodie der untersten Blüten der Blütenähre. Beim Vorkeimen der Knollen im Frühjahre 1957 wurden 0,2% mit Fadenkeimen festgestellt. Vor dem Lagern der Knollen im Herbst 1956 wurden 0,1% weichgewordene Knollen festgestellt. Im Jahre 1957 wurden schon Anfang Juli 0,2% „Kudrjašče" und bei 5,4% purple-top und yellow-top festgestellt. Von 95 Unkrautexemplaren von *A. a.*, die unter den Kartoffeln gewachsen waren, hatten 87 Blüten an den Gipfelpartien mit reduzierten Blütenkronen. Die in der Nachbarschaft der Kartoffeln unter der Zuckerrübe wachsenden *A. a.* waren noch völlig gesund, aber gegen Ende September wiesen doch zwei Pflanzen aus der Gesamtzahl von *A.-a.* in der Zuckerrübe Anzeichen viröser Vergilbung auf. Die in der Nähe in einem völlig geschlossenen Mistbeete wachsenden *A. a.*-Exemplare blieben bis zum Ende der Vegetation gesund. Am anderen Rande des Kartoffelfeldes wurden 20 Tomatenstauden ausgesetzt, von denen zu Ende der Saison 9, d. i. 45%, Merkmale des pseudoklassischen Stolburs aufwiesen. Die in einem Teile desselben Gartens wachsenden *A. a.*, die sich in einer Entfernung von 50—100 Meter befanden, abgetrennt durch Rasen und Wäldchen, blieben bis zum Ende der Vegetation gesund. Von Ende Juni an befanden sich im Garten und auf den Versuchsfeldern zahlreiche Zwergzikaden, vor allem *Empoasca flavescens* F., *Macrosteles laevis* Rib. und *Calligypona pellucida* F.

Die Virusinfektion wurde also auf *A. a.* durch Zikaden von kranken Kartoffeln übertragen, die Symptome von purple-top, yellow-top und „kudrjašče" aufwiesen. Infektionsquelle waren Knollen aus der Ernte des Jahres 1956. Später übertrugen die Zikaden die Infektion auch auf die Tomaten und die Unkrautstauden *A. a.* der nächsten Nachbarschaft, keineswegs aber an entferntere oder isolierte Orte.

Versuch Nr. 6. In den Kartoffelbestand des Versuches Nr. 5 wurden Anfang Juli, dann in der Hälfte und gegen Ende dieses Monats nacheinander je 25, 10 und 10 gesunde, im Glas-

Fig. 1. *Stellaria media* (L.) Vill., erkrankt an viröser Vergilbung. Blütenkronen vollkommen abortiert, Kelche hypertrophisch. — Foto J. Kubec.

Fig. 2. *Anagallis tenuifolia* L., als Indikatorpflanze für purple top und yellow-top der Kartoffeln in dem Versuch in Keřkov, im J. 1958. Blütenkronen der unteren Blüten reduziert, jene der oberen gänzlich abortiert. Samen haben sich entwickelt nur bei den untersten Blüten vor der Infektion. — Foto J. Kubec.

hause gezüchtete *A. a.*-Exemplare ausgesetzt. Von diesen 45 ausgesetzten *A. a.* zeigten im ganzen drei Pflanzen Anzeichen einer Vergilbungskrankheit und zwar zwei Pflanzen, die Ende Juli und eine, die Mitte dieses Monats ausgesetzt worden waren. Symptome waren samt und sonders pathologische Veränderungen der Blüten an den Stengelgipfelpartien.

Versuch Nr. 7. Es wurden im Glashause gleichfalls Drittel von Kartoffelknollen entsprechend dem Versuch Nr. 5 ausgesetzt und zwischen sie gesunde *A. a.* und *Stellaria media* (L.) VILL. Sie wuchsen in einem insektensicheren Glashause. Alle *A. a.* und auch *Stellaria* (L.) VILL. blieben gesund.

Versuch Nr. 8. Auf der Dachterrasse des Biologischen Instituts der Tschechosl. Akad. d. Wissenschaften wurden die verbleibenden Drittel der 400 Kartoffelknollen ausgesetzt, die in Versuch Nr. 5 und 7 verwendet worden sind. Das ausgewachsene Kraut wies Kennzeichen von „kudrjašče", purple-top und auch yellow-top zu gleichen Teilen wie bei Versuch 5 und 7 auf, aber wesentlich schwächer. Anfang Juni wurden hier Tomaten ausgesetzt, die mit Vergilbungsvirus mittels Pfropfreis von Ackerwinde (*Convolvulus arvensis* L.) *Anagallis arvensis* L. und *Stellaria media* (L.) VILL. infiziert worden waren. An den Tomaten befanden sich zahlreiche Blattläuse und *Trialeurodes vaporariorum* WESTW. Zwischen die Kartoffeln wurden am 9. Juli 10 und 18. Juli 20 gesunde *A. a.*-Exemplare ausgesetzt. Sowohl diese *A. a.*, als auch die aus Samen aufgewachsenen *A. a.* blieben bis zum Vegetationsende gesund, was auch für *Stellaria media* (L.) VILL. und für *Veronica chamaedrys* L., die ebenfalls aus Samen aufgewachsen sind, gilt. Gegen Ende Juli wurde neben den bereits erwähnten Blattläusen und *Trialeurodes* auch noch die Zikade *Empoasca flavescens* F. festgestellt. Von den Kartoffeln wurde die Erkrankung darum nicht übertragen, weil hier keine Zikaden vorhanden waren, welche Virosen aus der Gruppe des Stolbur übertragen und weil Blattläuse und *Trialeurodes* für diese Krankheiten keine Vektoren sind. *A. a.* kann darum nur dort ein Indikator von Virosen aus der Gruppe der Vergilbungskrankheiten und des Stolbur sein, wo sich auch ihre Überträger befinden.

III. Teil

Versuch Nr. 9. Versuchsstelle ist Dol bei Libčice, auf einer Lokalität, auf welcher schon vom Jahre 1954 an klassischer Stolbur festgestellt worden ist („Dol-Stamm") mit typischen Symptomen der Hypertrophie der Kelche bei den Tomaten (BLATTNÝ et al. 1956). Hier konnte man charakteristisch erkrankte Exemplare von Ackerwinde [*Convolvulus arvensis* L.,) *A. a.*, *Stellaria media* (L.) VILL., *Veronica* sp., *Reseda* sp. in je nach den Jahren schwankender Menge finden. Hier wurde ebenfalls der klassische Vektor des Stolburs, die Zikade *Hyalesthes obsoletus* SIGN. in einer Intensität festgestellt, die den einzelnen Jahren entsprach.

Im Jahre 1957 wurden hier die Anzeichen der europäischen aster-yellows bei einer ziemlich grossen Anzahl von Pflanzen festgestellt, beispielsweise bei ca 30% *Gaillardia amblyodon* GAY, 5% *Delphinium ajacis* L., 20% *Antirrhinum majus* L., 20% *Scabiosa atropurpurea* DESF., 5—10% *Callistephus chinensis* (L.) NESS., 15% *Centaurea americana* NUTT., 10% *Reseda odorata* L., 10% *Anchusa capensis* THUNG., 5% *Malope trifida* CAV. *grandiflora hort.*, 15% *Eschscholtzia californica* CHAM., 100% Blumenkohl, *Convolvulus arvensis* L., und *A. a.* mit schweren Kennzeichen der Virose. Bei Tulpen, Krokussen und Anemonen, die von anderer Stelle her versetzt worden sind, wurden keinerlei Krankheitszeichen festgestellt.

Im Jahre 1958 sank der Anteil an derartig erkrankten Pflanzen auf ein Minimum: *Callistephus* sp. 0,1%, *Gailardia* sp. 0,3%, *Eschscholtzia* sp. 0,6%, *Gypsophila elegans* BIEB. 2,6%, die übrigen ohne Anzeichen. Die Ackerwinden blieben hier auch weiter virös, der Anteil an erkrankten Pflanzen war um 50% niedriger, als im Jahre 1957. *A. a.*-Exemplare, die zwischen den Ackerwinden wuchsen, erkrankten im Jahre 1958 an typischer, viröser Vergilbung, jedoch nur in 10% der Anzahl des Jahres 1957.

Im Jahre 1954 zeigten Tomaten, die hier unter die kranken Ackerwinden gesetzt worden waren, zu 10% die gleichen Anzeichen klassischen Stolburs („Dol-Stamm"). Im Jahre 1958 wurden hier 100 Tomatenstauden gesetzt und von ihnen erkrankte 1% unter Anzeichen des pseudoklassischen Stolburs („Loděnicer Stamm"). Die Identität der Erkrankung, wie sie sich an den hier angeführten Zierpflanzen zeigte, mit dem klassischen Stolbur, ist bisher noch nicht erwiesen worden. Im Jahre 1957 gab es hier viele Zikaden *Macrosteles laevis* RIB. und *Calligypona pellucida* F., bei denen erwiesen worden ist, dass sie Überträger der Viren dieser Gruppe sein können (HEINZE et al. 1955, BLATTNÝ 1957, MUSIL und VALENTA 1958).

IV. Teil

Versuch Nr. 10. Versuchsort: Loděnice bei Beroun. Auf einer Lokalität, auf der sich *Convolvulus arvensis* mit Anzeichen von Stolbur befand, wurden im Jahre 1954 20 Tomatenstauden ausgesetzt, von denen 10% Symptome des pseudoklassischen Stolburs zeigten („Loděnicer Stamm", Blattný et al., 1956). In den Jahren 1956 und 1957 wuchsen ständig auf dieser Lokalität kranke Exemplare von *Convolvulus arvensis* L., *A. a.* und zwar sowohl die blaue wie die rote Form sowie (weniger) erkrankte *Stellaria media* (L.) Vill. und *Veronica* sp. In der Zikadenpopulation, die vom Jahre 1954 an verfolgt wurde, herrschten *Macrosteles laevis* Rib. und *Calligypona pellucida* F. vor. *Hyalesthes obsoletus* Sign. wurde hier trotz zahlreicher Versuche, diese Art zu fangen, bisher nicht gefunden.

In der Hälfte Mai 1958 wurden hier zwischen die kranken *Convolvulus*-Exemplare 100 Tomaten ausgesetzt. Anfang September wurden bei 1% Tomaten Symptome des pseudoklassischen Stolburs mit nahezu samenlosen Früchten festgestellt. Die Zahl der Zikaden dieses Jahres sank auf ein Zehntel des Standes vom Jahre 1957 und das Vorkommen war gleichfalls um drei Wochen verspätet. Auch die Zahl der kranken Exemplare von *Convolvulus arvensis* L. und *A. a.* sank um ein Drittel. resp. vier Fünftel.

V. Teil

Versuch Nr. 11. Versuchsstelle: Garten der phytopathologischen Abteilung des Biol. Inst. der Tschechosl. Ak. d. Wiss., Praha-Dejvice. Auf dem Felde wurden Kartoffelknollen mit Fadenkeimen ausgesetzt. Während der Vegetation zeigten 2,9% von ihnen Symptome von purple-top und yellow-top. In der Nachbarschaft befand sich ein Maisbestand und in demselben wuchsen aus Knollen, die im Boden überwintert waren, auch Kartoffeln. In beide Kartoffelbestände wurden am 5. und 11. Juni im ganzen 50 gesunde *A. a.*-Exemplare ausgesetzt und weitere 16 wuchsen dort aus Samen auf. Bis zum Ende der Vegetation äusserte sich nur bei zwei Exemplaren von *A. a.* eine viröse Erkrankung und zwar in Form zerfranster und reduzierter Kronen der Gipfelblüten. Gegenüber dem Jahre 1957 war der Prozentanteil der erkrankten Pflanzen weit niedriger, obwohl die Zusammensetzung der Zikadofauna die gleiche geblieben ist. Nur 2% der Tomatenpflanzen, die in nächster Nachbarschaft wuchsen, erkrankten an pseudoklassischem Stolbur zum Unterschied von 45% des Jahres 1957.

Versuch Nr. 12. Versuchstelle: Kartoffelzüchtungsstation Keřkov, 540 m über dem Meeresspiegel. Zwischen den Kartoffelbestand wurden 85 gesunde Exemplare *A. a.* und 15 *A. linifolia* L. (*A. grandiflora* hort.) ausgesetzt. Ende August wiesen 59 Exemplare *A. a.* und alle *A. linifolia* L. Symptome der typischen Virose auf — Fransigkeit und später völlige Reduktion der Blütenkronen. Ende Juli wurden hier weitere 25 *A. linifolia* L. ausgesetzt, die bis Ende August noch nicht blühten und bereits deformierte Gipfelblätter aufwiesen. Durch die Kartoffelernte wurden diese Pflanzen vernichtet. Von Zikaden waren in den Pflanzenbeständen besonders folgende Arten anwesend: *Empoasca flavescens* F., *M. laevis* Rib., und *C. pellucida* F., schwächer *Euscelis plebejus* Fall. sowie *Aphrodes bicinctus* Schrk. *Anagallis linifolia* L. erwies sich hier als Indikator der Anwesenheit von Viren aus der Gruppe der Vergilbungskrankheiten und des Stolburs genau so empfindlich, wie *A. a.* Auf die Empfindlichkeit dieser Art gegen den Komplex der Vergilbungskrankheiten machte mich Ing. C. Blattný jun. aufmeksam, der diese Art im Jahre 1957 im Libochovicer Gebiete stark an dieser Virose erkrankt vorfand. Im Kartoffelbestand in Keřkov zeigte etwa 1% Pflanzen Ende Juni die Symptome von purple-top und yellow-top, die offensichtlich an *A. a.* und auch *A. linifolia* L. indiziert worden sind. In weiterer Umgebung des Versuchsfeldes trat Phyllodie bei *Trifolium repens* L. und *T. pratense* L. auf, deren Verhältnis zu purple-top und yellow-top nicht näher bestimmt, aber sicher sehr eng ist, wie die Arbeiten von Web und Shultz (1955) an einer anderen Kleeart in der Beziehung der amerikanischen Vergilbungskrankheit der Astern zum purple-top bei Kartoffeln andeuten. Auf den Unterschied der Virus-Arten oder Stämme, die bei uns an Klee Phyllodie hervorrufen, vom Virus des Stolburs weist Valenta (1958) hin.

Versuch Nr. 13. Versuchslokalität: Kartoffelfeld, 4 Hektar, Sorte „Krasava", im Kataster Mikulov in Mähren. Ein typisches Vorkommensgebiet des klassischen Stolbur an allen Solanaceen, vor allem Kartoffeln, Tomaten, Paprika und Tabak in den Jahren 1952—56. Das Kartoffelpflanzgut stammte aus dem Böhmisch-Mährischen Hügelland. In der Zeit der Vege-

tation sind in diesem Bestande weder Symptome von Stolbur, noch von purple-top oder yellow-top festgestellt worden. Vergilbungsartige Erkrankungen des Typs aster-yellows an *Cirsium arvense* Scop. und *Carduus acanthoides* L., sowie an anderen Pflanzen fanden wir dort nur selten, aber *A. a.*, sowohl die rotblütige, als auch die blaublütige Form, war dort in den vorhergehenden Jahren typisch virös. Im J. 1958 wurde in der Umgebung weder Stolbur noch Vergilbung festgestellt.

In den Kartoffelbestand wurden Ende Mai an verschiedenen Stellen 120 gesunde *A. a.* und 15 gesunde *A. linifolia* L. ausgesetzt. In der Julihälfte wurden weitere 78 Exemplare *A. a.* ergänzend gesetzt. Infolge der Trockenheit und anderer ungünstiger Bedingungen gingen 99 der ausgesetzten *A. a.*-Pflänzchen und alle *A. linifolia* L. zugrunde. Von den restlichen 99 *A. a.* äusserte Ende August nur eine einzige Pflanze viröse Symptome, blassere und kleinere Blütenkronen, mässig gewellte oberste Gipfelblätter und das Rosigwerden der Blattränder der Grundblätter. Es handelt sich ohne Zweifel um eine Infektion, die von Zikaden von irgendeiner an irgendeinem Virus der Gruppe der Akarpien erkrankten Pflanze der Umgebung übertragen worden ist. Von Zikaden trat auf dieser Lokalität in den vergangenen Jahren sehr zahlreich *Hyalesthes obsoletus* Sign. auf. Im Jahre 1958 wurden hier bloss *Calligypona pellucida* F., *Macrosteles laevis* Rib. und *Euscelis plebejus* Fall. festgestellt.

Diskussion

Wie im Jahre 1957, so indizierte *A. a.* auch im Jahre 1958 (das für Virosen dieser Art ungünstig war) die Anwesenheit von Viren dieser Gruppe in einer bestimmten Lage nach den bisherigen Versuchen exakter die Anwesenheit von Stämmen des sog. pseudoklassischen Stolbur (einschliesslich der europäischen aster yellows, purple-top und yellow-top an Kartoffeln) als die Anwesenheit des klassischen Stolbur. Aber auch dort, wo der klassische Stolbur häufig vorkommt, ist in der Regel auch die Virose von *A. a.* häufig; und Stolbur lässt sich durch die Zikade *Euscelis plebejus* Fall. auf *A. a.* übertragen und an ihr ruft er die typischen Kennzeichen der von uns beschriebenen Virose hervor. (Valenta, Musil 1958). Die Sachlage ist wahrscheinlich noch komplizierter. Der Grund kann rein trophisch sein, so ziehen zum Beispiel die Zikadenarten bestimmte Nährpfanzen in der oder jener Lage vor. In Hustopeče bei Brno, in sehr warmer Lage wurde in den Jahren 1952—56 auch massenhaftes Auftreten von klassischem Stolbur beobachtet, z. B. auch das Welken der Kartoffeln. Sehr häufig fanden sich hier an *Cirsium arvense* Scop. die Kennzeichen des sog. europäischen aster yellows. Im Jahre 1957 fanden sich hier Symptome der Virose an *A. a.* nur stellenweise häufiger. Im Jahre 1958 gab es hier Ende August nur sehr wenig kranke Exemplare von *Cirsium arvense* L., an Kartoffeln und anderen Pflanzen im Jahre 1958 jedoch keine Symptome klassischen Stolburs. Unter vielen Hunderten von Exemplaren von *A. a.* wurde nur eine einzige Pflanze mit sehr leichten Anzeichen einer Virose gefunden. Im Jahre 1958 konnte es nur zu einer kleinen Zahl von Infektionen infolge des Charakters des Wetters und des so verursachten schwachen und verspäteten Auftretens von Zikaden kommen. Dennoch bleibt die kleine Zahl erkrankter *A. a.*-Exemplare in dieser Lage bisher unerklärlich. Dabei schliessen wir die Möglichkeit einer selektiven Fähigkeit bestimmter Zikadenarten nicht aus, nur manche Viren, oder Viren-Stämme zu übertragen (Blattný 1958c).

Herde für Stolbur und andere Krankheiten dieser Gruppe können aller-

dings auch andere sein, als die, welche wir bisher kennen gelernt haben. Bei uns — in Hustopeče, Loděnice und anderswo — wie auch im Auslande (Nyon in der Schweiz) fanden wir den Vogelknöterich (*Polygonum aviculare* L.) mit gelblichen bis rosig angehauchten und verkleinerten Gipfelblättern und einer verringerten Blütenzahl, oder überhaupt nicht blühend oder keine Samen erzeugend. Solcher Art erkrankte Pflanzen fanden sich in der Nähe von Ackerwinden und anderen Pflanzen, die von Stolbur oder verwandten Erkrankungen befallen waren. Kvíčala (1958) machte als Erster darauf aufmerksam, dass es sich bei dieser Pflanze um eine Erkrankung durch das Stolbur-Virus oder ein mit ihm verwandtes Virus handeln könnte. Ähnlich kann das auch beim Breitwegerich (*Plantago major* L.) sein. In Lagen, in denen bei uns die Virose der *A. a.* stark auftritt, fanden wir bei dieser Pflanze Phyllodie der unteren Blüten in der Blütenähre, mässig gewellte Blätter mit hervortretenden Adern auf der Unterseite und manchmal anthokyanisiert. Unsere Versuche weisen auf die Übertragbarkeit dieser Virose auf Kartoffeln und auch *A. a.* hin. (Blattný 1959). In Norditalien fand Grancini (1957) in Lagen, in denen er bei verschiedenen Pflanzen ein reiches Auftreten der europäischen aster-yellows konstatierte, auch sehr häufig hexenbesenförmigen Wuchs bei Plantago major L. und Virescenz der Blüten bei *Plantago lanceolata* L. In Nyon in der Schweiz (Changins) tritt der klassische Stolbur selten bei Tomaten auf (Bovey 1956). Bei meinem Besuch dortselbst im Jahre 1957 sah ich, dass es dort nirgends ein stolburkrankes Exemplar von *Convolvulus arvensis* gab. Nirgends habe ich *A. a.* gefunden, weder gesund noch krank. Sehr häufig und schwer erkrankt an Phyllodie fand sich dort nur *Plantago major* L. vor. Man kann dafürhalten, dass diese Pflanze hauptsächlich als Ansteckungsherd fungierte. Es gibt Lagen, in denen Kartoffeln und Tomaten an typischem klassischem Stolbur erkrankt vorkommen, während *A. a.* grösstenteils an ihrer eigenen, typischen Virose krankt und Lagen, in denen Kartoffeln und Tomaten von klassischem Stolbur befallen sind, während *A. a.* grösstenteils gesund geblieben ist und Lagen, in denen bei ähnlich erkrankten Kartoffeln und Tomaten *Stellaria media* (L.) Vill. oder *Veronica* sp., nicht aber *A. a.* an Virosen gleichen Typs stark leiden. Analog gilt diese Verschiedenheit auch für Lagen, in denen sowohl Stolbur als auch Erkrankungen vom Typ der europäischen aster-yellows auftreten. Das gilt auch von Lagen — wie es beispielsweise das Vorbergland der Slowakei ist — in denen der klassische Stolbur nicht auftritt, wohl aber ausschliesslich die Erkrankungen vom Typ der europäischen aster-yellows. Wir erachten diese Vorstellungen für die Konsequenzen in einer experimentellen Arbeit schwerwiegend, die vor allem überzeugend feststellen sollte, ob es sich

a) um Virusstämme handelt, die stabilisiert sind oder neu entstehen und unter welchen Bedingungen (z. B. inbezug auf die Temperatur, auf die Wirtsorganismen, ob es sich nun um einen tierischen Vektor oder um einen pflanzlichen Wirtsorganismus handelt);

b) Virusstämme, die lokal, d. i. geographisch stabilisiert oder veränderlich sind;

c) ob es Virusstämme gibt, welche in der oder jener Lage mehr oder minder an bestimmte Wirtsorganismen gebunden sind;

d) ferner, wie die gegenseitigen Beziehungen des Stolbur und seiner Stämme

sind, des pseudoklassischen Stolbur und der Vergilbungskrankheiten verschiedener Stämme, ihre Koexistenz und Inkompatibilität in Wirtspflanzen und Vektoren;

e) schliesslich, wie die Beziehung der einzelnen Zikadenarten — vor allem trophische — zu verschiedenen Wirtspflanzen ist.

Anagallis arvensis L. resp. *Anagallis linifolia* L. indizieren die Anwesenheit dieser Virengruppe in einer bestimmten Lage. Es gibt Fälle, in denen es zur Übertragung der Viren nicht anders kommen konnte, als von Kartoffeln (siehe Versuche in Třebotovice und Keřkov). Nicht immer aber indizieren *A. a.* und *Anagallis linifolia* L. die Gegenwart dieser Viren in einem bestimmten Kartoffelbestand (siehe beispielsweise Versuch in Mikulov): Auf *A. a.* könnten diese Krankheiten auch von anderen, insbesondere von wildwachsenden Pflanzen anderer Art übertragen worden sein. Bei der grossen Beweglichkeit der Zikaden und auch bei ihren passiven Bewegungen bei Wind kann so etwas verhältnismässig leicht geschehen. Wir kennen Lagen (Praha-Břevnov), in welchen *Convolvus arvensis* L. an diesen Virosen nicht krankt und nicht einmal kranke Pflanzen von *Stellaria media* (L.) VILL. zu finden sind, die das Virus über den Winter bewahren könnten. Es handelt sich um abgesperrte Gartenflächen, in die auch ein Zuflug von Zikaden aus anderen, entfernteren Lagen wenig wahrscheinlich ist. In solchen Lagen waren in den Jahren 1956 und 57 diese Virosen an Tomaten häufig: Von 60 Pflanzen des Jahres 1957 waren dort an pseudoklassischem Stolbur 20 Pflanzen (33%) erkrankt; die Virosen waren weiter häufig an *A. a.*, *Stellaria media* (L.) VILL., weiter an Zierpflanzen, *Callistephus chinensis* (L.) NESS, *Chrysantheum coronarium* L., *Scabiosa atropurpurea* DESF. und an *Allium ampeloprasum* L. subsp. *porrum* (L.) REGEL. Auch im Jahre 1958 trat diese Viruserkrankung auf, jedoch zu sehr kleinem Anteil an *A. a.* und an 4% Tomaten. Wir erklären uns dieses Auftreten durch die Persistenz der Viren in den Zikaden: *Calligypona pellucida* F. überwintert in ihren Nymphen, bei *Macrosteles laevis* RIB. ist die Form der Überwinterung noch nicht genügend bekannt (bisher wurde festgestellt, dass diese Art als Ei überwindert). Wir schliessen nicht einmal eine transovariale Übertragung der Viren aus. Und als Überträger kommen vielleicht noch andere, bisher nicht festgestellte Zikadenarten in Betracht.

Unsere Versuche zeigten, dass bei dem Versuche des Jahres 1957 in Praha-Dejvice die Virose nicht von *A. a.* auf die Kartoffeln, sondern von den Kartoffel auf *A. a.* überging. Das erwies eigentlich erst der Versuch des Jahres 1958, da auf diesem Felde am 23. 8. 1958 genug Kartoffeln mit Merkmalen von purple-top und yellow-top vorgefunden wurden, die zwischen ihnen wachsenden Pflanzen von *A. a.* bei sehr kleiner Zikadenmenge jedoch fast völlig gesund blieben. Wie im Jahre 1957, so gab es auch im Jahre 1958 hier aus den früheren Jahren genug virentragende Zikaden, auch in der Umgebung fanden sich virenbeherbergende Pflanzen vor, *Stellaria media* (L.) VILL., *Plantago major* L. aber diese Herde reichten offenbar zur Ansteckung nicht aus. Infolge des kühlen und regnerischen Frühjahrs und der ersten Sommerhälfte des Jahres 1958 traten die virophoren Zikaden in weitaus geringerer Zahl und später, als im Jahre 1957 auf. In Mittelböhmen lag ihr Massenauftreten um 14 Tage bis drei Wochen später und war um $^9/_{10}$ schwächer als im Jahre 1957. In der Umgebung von Prag und an anderen mittel-

böhmischen und wärmeren Lokalitäten wurden auch die Symptome der Virose bei *A. a.*, mindestens um 14 Tage später, sichtbar, als im Jahre 1957: Im Jahre 1958 Ende Juli, im Jahre 1957 Anfang Juli. Der Anteil an kranken *A. a.*-Exemplaren des Jahres 1958 sank im Vergleiche zu 1957 bedeutend, an manchen Stellen ganz wesentlich. Es handelte sich also um einen für die Verwendung von *A. a.* als Indikatorpflanze für die Gegenwart der Viren der Gruppe der Samenlosigkeit weit ungünstigeren Jahrgang.

Aus diesen Beobachtungen und Versuchen gehen einige Bemerkungen zur Ökologie der Viren hervor:

1. Das Auftreten von Virosen dieser Gruppe schwankt in den einzelnen Jahrgängen insbesondere bei einjährigen Pflanzen stark: Nach einem Jahr des allerstärksten Auftretens kann unter unseren Bedingungen ein Jahr sehr schwachen Auftretens folgen (1957: stark, 1958: sehr schwach).

2. Die Gründe für dieses Schwanken können äussere und innere sein, diese Viren (der Samenlosigkeit-Akarpie, ob es sich nun um Stolburstämme, den pseudoklassischen Stolbur oder die Gruppe aster-yellows handelt) überwintern bei uns in Pflanzen [*Convolvulus arvensis* L., *Stellaria media* (L.) Vill., und sicher auch in vielen anderen]. Sie überwintern auch in Zikaden (*Calligypona*, *Hyalesthes*). Diese beiden Initialquellen, diese Glieder der Infektionskette sind zwar für die Erhaltung der Art bedeutsam, aber nicht entscheidend für eine Übervermehrung in der nächsten, nachfolgenden Periode. Für die Vermehrung des Virus sind weitere Bedingungen entscheidend. Darin stimmt die Übervermehrung der Viren mit den für andere Organismen geltenden Gesetzmässigkeiten überein. Unter die äusseren Bedingungen, die eine Übervermehrung des Virus, sein starkes Auftreten und seine Schädlichkeit bei Pflanzen bewirken, gehört die Menge und Zeitigkeit der Zikadofauna, welche diese Viren überträgt. Eine praktische Applikation dieses Zusammenhangs ist für eine Signalisation sehr bedeutsam.

Am allerunklarsten sind die inneren Bedingungen, wie beispielsweise die Empfindlichkeit verschiedener Pflanzenarten für eine Erkrankung an diesen Virosen. Eine Sammlung der Tatsachen, in der letzten Zeit verbreitet, genügt nicht. Umsoweniger genügt unsere Kenntnis, soweit sie die Empfindlichkeit der Pflanzen in ihren verschiedenen Wachstumsphasen betrifft: die erhöhte Empfindlichkeit der jungen Pflanzen scheint hier keine Allgemeingiltigkeit zu besitzen. Bei diesen Viren ist auch das Schwanken ihres Spiegels in den Pflanzen wichtig und eine Veränderung der Inkubationsdauer im Zusammenhang mit der ontogenetischen Entwicklung der Wirtspflanze (Blattný 1959).

3. Bedeutsam scheint auch der Einfluss der Wärme und zwar sowohl auf das Virus in den Zikaden, als auch in den Pflanzen zu sein. Kunkel (1937) hat festgestellt, dass das Virus der amerikanischen aster-yellows in den Zikaden praktisch schon bei 32° C inaktiviert wird. (Ich lasse Zweifel, die später über diese Beobachtung geäussert worden sind, beiseite.) Im Jahre 1957 erkrankten bei Versuchen in Prag in der Karlovka *A. a.*-Exemplare, die im Frühjahre aus Samen zwischen Kartoffeln wuchsen, die von purple-top und yellow-top befallen waren, zu 95,1% an Vergilbung. Die am 10. 7. 1957 und danach in den gleichen Bestand gesetzten *A. a.*-Exemplare, die also nach der Periode des letzten Junidrittels 1957 ausgesetzt waren, als hier die Temperaturen 35° C im Schatten erreichten, erkrankten nur zu 6,6%. Das sehr

schwache Vorkommen des Stolburs und verwandter Akarpien in Südmähren und der Südslowakei im Jahre 1958 liess sich nicht nur durch die Verspätung und Geringfügigkeit des Vorkommens der Zikaden erklären, sondern auch durch die ausserordentlich warme und trockene Temperaturperiode im Mai und Juni 1958 in Mikulov (am 11. V. 32° C, am 3. VI. 28,1° C!), welche sich virostatisch äussern konnte. Die steigende Intensität der Erkrankung von *A. a.* und Verwandten an Akarpien im Spätsommer und im zeitigen Herbst spricht von einem günstigen Einfluss niederer (aber nicht zu niedriger) Temperaturen auf die Vermehrung der Viren. Für die gemässigten Ansprüche an den Wärmeverbrach spricht bei Stolbur auch diese bekannte Tatsache: Das starke Auftreten von Stolbur in den kühleren (dabei aber vom mitteleuropäischen Standpunkt aus noch immer warmen) Teilen der Bulgarischen Volksrepublik und das schwache Autreten dieser Virose in ihren sehr warmen Gebieten. Der inaktivierende Wärmepunkt für das Virus des Stolbur und verwandter Erkrankungen liegt nicht allzuhoch. PANJAN (1958) stellte eine günstige thermotherapeutische Wirkung auf das Stolburvirus in Tomatenreisern bei 43° C und einer Wirkungsdauer von 24 Stunden fest. Weitere genauere Berichte über Ergebnisse von Versuchen, die in dieser Richtung vorgenommen worden sind, ob es sich nun um eine virostatische Wirkung höherer Temperaturen oder um eine Rehabilitation des Gesundheitszustandes der Pflanzen, in anderem Zusammenhange erworben, handelt, sind in Kürze zu erwarten (VALENTA 1958). Schon ENGELMANN's Feststellung (1832) von einem stärkeren Auftreten pathologischer Blütenformen (von denen wir heute wissen, dass sie infolge von Virosen verändert worden sind) in feuchteren Jahren zeugt vom Einfluss des Milieus, das für die Viren dieser Gruppe über alles wichtig ist.

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Anagallis arvensis L. как показатель присутствия вирусов из группы акарний — столбуров и желтух

ЦТИБОР БЛАТТНЫ

Резюме

Желтуха *Anagallis arvesis* L. свидетельствует, по нашим наблюдениям, о присутствии в природе вируса из группы акарний (желтух и столбуров). *Anagallis arvensis* L., и *Stelaria media* (L.) Vill. указывают на присутствие в опытах в 1957 г. этих болезней, и также purple-top, yellow-top у картофеля в том случае, если были в наличии *Macrosteles laevis* Rib., и *Calligypona pellucida* F. Опыт на зостекленной террасе при отсутствии обоих выше названных видов показал, что переносчиком приведенных заболеваний не могут быть цикадки *Empoasca flavescens*, тли различных видов, белокрылка *Trialeurodes vaporariorum* Westw. В 1958 году, когда численность цикадок была ниже и они появлялись позже, приведенные растения, а также *Anagallis linifolia* L., указывали на присутствие этих вирусов как на картофельных полях (puple-top, yellow-top даже в предгорьях), так и в других растительных очагах (растениях резервуарах), или в цикадках, но слабее и позднее чем в 1957 г. *Anagallis arvensis* L., *A. linifolia* L. в некоторых местах точнее свидетельствуют о присутствии aster yellows (европейской желтухи астр) и псевдоклассического столбура, а на других местах группы классического столбура; причины этого явления до сих пор неизвестны. Желтуха, перенесенная цикадками с *Anagallis arvensis* L. на томаты проявлялась на них как псевдоклассический столбур. Yellow top картофеля, желтухи и классический столбур, перенесенные цикадками на *Anagallis arvensis* L. проявлялись здесь типичным недоразвитием лепестков. Сильное колебание в распространении этих вирусов в природе (после 1957 г., когда часто встречались желтухи в Средней Чехии, последовал год со слабой заболеваемостью) зависит от начального источника инфекции, от времени, когда начинают появлятся и размножаться цикадки, далее от инактивирующего действия высоких температур, и также от других причин (колебания в численности вирусов в растениях и т. п.).

Растения *Anagallis arvensis* L., *Anagallis linifolia* L. и *Stellaria media* (L.) Vill. могут служить индикаторами, указывающими на присутствие вирусов из группы акарний в данном биотопе. Наблюдения над составом и фенологией цикадок фауны может служить одним из методов сигнализации мер борьбы против этих вирусных заболеваний.

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Oat Sterile-Dwarf Virus Disease

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Souhrn

V práci jsou shrnuty nové důkazy o virovém charakteru sterilnosti a zakrslosti ovsa (VSZO), jakož i stručné zmínky o zjištění dalšího viru v ČSR — virové proužkovitosti pšenice (VPP). Oba viry mají stejného přenašeče — křísa *Calligypona pellucida* F. Virus proužkovitosti pšenice může být na rozdíl od VSZO předáván nakaženými samicemi vektora na potomstva transovariálně. Tato vlastnost, jež umožnila vzájemné oddělení obou virů, byla tím prokázána u fytopathogenních virů na evropské pevnině prvně. VSZO může být přenášen larvami i imagy křísa. Značně variabilní cirkulační doba VSZO v těle vektora proběhne většinou za 3 až 4 týdny. Inkubační doba VSZO činila u ovsa 3 až 4 týdny, u pšenice a ječmene 4 až 7 týdnů; žito projevovalo za 4 týdny pouze zaostávání v růstu. K přenesení infekce byl nutný minimálně půlhodinový pobyt vektora na testační rostlině; po třídenním pobytu došlo již ke 100% infekci. Destruktivita VSZO na hostiteli se nezvětšuje úměrně ani s délkou sání křísa, ani s přibývajícím počtem vektorů na rostlinu. Koncentrované extrakty z rozdrcených těl nakažlivých křísů aplikované mechanickým vtíráním i injekcemi do ovesných rostlin nezpůsobily u nich chorobné symptomy. VSZO ani VPP se nepodařilo přenést půdou nebo kokoticí. VSZO byl však přenesen roubem. Oba viry vyvolaly charakteristické symptomy u *Avena fatua* L. a *Poa annua* L. Zjištěné skutečnosti diskutovány z etiologického hlediska.

Summary

This paper presents a summary of new evidence for regarding sterility and dwarfing of oats (OSDV) as a virus disease. Brief references are also made to the identification of a further virus in Czechoslovakia—the wheat striate virus (WSV). Both viruses are transmitted by the leafhopper *Calligypona pellucida* F. The wheat striate virus differs from the oat sterile-dwarf virus

in that it can be passed transovarially by the infected females of the vector to their progeny. This characteristic, which made it possible for the two viruses to be separated from each other, was demonstrated for the first time for pathogenic viruses on the European continent in this case. OSDV can be transmitted by leafhopper larvae and adults. The time during which OSDV circulates in the vector's body is very variable; it is usually three to four weeks. The incubation period of OSDV was three to four weeks for oats, four to seven weeks for wheat and barley; rye only showed retarded growth after four weeks. For infection to take place it was necessary for the vector to remain for a minimum of half-an-hour on the test plant; 100% infection was obtained when the vector remained on the plant for three days. The injury inflicted by OSDV on the host does not increase proportionately either with the duration of the feeding period or with an increase in the number of vectors on the plant. Concentrated extracts of the crushed bodies of infectious leafhoppers produced no symptoms of disease, when rubbed or injected into oat plants. Nor were attempts to transfer OSDV and WSV through soil or dodder successful. OSDV was, however, transferred by grafting. Both viruses gave rise to characteristic symptoms in *Avena fatua* L. and *Poa annua* L. These findings are discussed from the etiological point of view.

Introduction

The significance of virus diseases in European cereals and fodder grasses has increased considerably in recent years, and numerous reports have appeared on the subject (Klinkowski and Kreuzberg 1958). The economic losses caused by this category of disease are of such magnitude that they can no longer be underestimated. Czechoslovakia may serve as an example of one of the countries in which virus on cereals had not been definitely identified up to 1957.

For several decades, however, the periodic occurrence of very harmful diseases on oats had been observed in south-east Bohemia. The disease appeared in some years and there seemed also to be some correlation with climatic factors. Until recently the causes of the disease were sought among various agents both of a parasitic and non-parasitic nature. In 1943 Blattný, after examination of oat crops near Pelhřimov, expressed the view (v. also Bojňanský and Blattný 1956) that the symptoms he observed might be due to a virus. He repeated this at the conference at Liblice in the winter of 1951/2.

The systematic study of the etiology of oat disease did not begin until the epidemic was spreading with unprecedented rapidity in 1954 to 1956, when losses reached catastrophic proportions. The only measure for resisting the disease, of course a provisional one, was a ban on the cultivation of oats in the threatened districts of the Jihlava region.

The authors of the present paper first met with the disease in July 1956. On the basis of an analysis of the symptoms, type of distribution and gradology of the disease, and from data on the occurrence of probable vectors,

the most likely cause was considered at that time to be a virus of the yellows type (PRŮŠA 1957). During the 1957 vegetative period it was so far possible to support the correctness of this conception that the actual originator of the calamity could be described as a new virus (PRŮŠA 1958a, 1958b). This virus was found to have characters distinguishing it from those viruses which could be considered on the basis of certain features to be related (PRŮŠA 1958b).

Oat sterile-dwarf virus disease (OSDV), as the new disease has been named (PRŮŠA 1958b), is not, however, confined only to Czechoslovakia. In the authors' opinion the same virus has been causing increasing damage in the Scandinavian countries, particularly since 1949, especially in Finland, where it caused an even greater catastrophe than in Czechoslovakia (KANERVO et al. 1957). It was in Finland, also, that the connection between the distribution of the disease and the leafhopper *Calligypona pellucida* F. was first established (HEIKINHEIMO 1956, 1957; KANERVO et al. 1957). The specific relation between this leafhopper and the spreading of this dangerous disease was experimentally confirmed shortly after, and independently of the Finnish workers, in Czechoslovakia (DLABOLA 1957, 1958; PRŮŠA 1958a, 1958b). According to some as yet unconfirmed reports, a similar disease has also occurred in Slovakia in the Orava basin. BLATTNÝ (1958) discovered a symptomatologically similar type of disease in Rumanian Transylvania.

Although the nature of the pathogenic agents of sterility and dwarfing in oats has been studied in its main outlines, it was still necessary to provide further and still more convincing evidence in order to dispel any doubt as to its virus origin. This has been the main aim of the work here presented.

At the same time the presence of another virus was determined. A detailed report on this will be given separately. This virus produces, in addition to dwarfing and other less obvious symptoms, a marked striation in cereals (PRŮŠA 1958b), which is not produced by OSDV. It has been named, according to the most characteristic symptom on the host where it was first found, wheat striate virus.

These two viruses often occur together, which is connected with the fact that they have the same vector (PRŮŠA 1958b). WSV is not of great economic importance so far; not perhaps because it is less injurious to the host cereals than OSDV, but because it has not yet occurred on a mass scale. It is, however, theoretically noteworthy for its behaviour in the vector, the infected females of the leafhopper *Calligypona pellucida* having the ability of transmitting it to their progeny transovarially, which is the first case of this kind to be demonstrated for a phytopathogenic virus on the European continent. This virus is probably identical with that described under the name "European cereal (wheat) striate mosaic" in recent times in England, and diseases observed in Germany and Denmark (SLYKHUIS 1958) probably belong to it too.

Material and Methods

In order to elucidate the question of the existence or non-existence of toxic and non-toxic populations of the leafhopper *Calligypona pellucida* it was necessary to substitute the simpler practice of mass transfer of leafhoppers to whole groups of test plants by a method of caging them individually on the plants, one insect on one plant. In this way it is possible to follow the pathogenic power of each leafhopper independently throughout the postembryonal development up to its death in a series of passages on individual test plants (v. fig. 1 and 2). The leafhoppers which were shown by sufficiently prolonged preliminary testing on healthy plants to be virus free could then be selected and used in later phases of work for experiments with their artificial infection. The uninfected insects were divided into two groups, experimental and control. Individuals from the experimental group were caged on diseased plants for a certain time for acquisition feeding. They were then transferred at definite time intervals on to a series of test plants in order to establish whether they had acquired infective power and, if so, how long after the period of feeding from the source of infection. The insects from the control group were put through a similar process with the difference that they were never brought into contact with infected plants. During the time when the experimental insects were feeding on infected plants the controls were isolated on healthy plants.

Using the method described it was possible to determine whether leafhoppers are able to become infected by feeding on diseased plants. This was a matter of primary importance in this investigation, since if the leafhoppers became infected under these circumstances it could not be explained as being due to toxins, because toxins differ from viruses in not being infectious. The experimental procedure described also ensured that in the event of results being positive it would be possible to obtain information on the length of the circulation period of OSDV in the body of the vector.

Results

Determination of the degree of natural infection of leafhoppers and artificial infection of virus-free adults of *Calligypona pellucida*

Experiments were started at the beginning of April 1958 in the glasshouse and were continued from May to about the middle of July in a frame made of hotbed windows in which the glass had been replaced by light silon material, allowing the insects and plants to develop under natural conditions. One hundred and sixty larvae in the hibernating stage of the leafhopper *Calligypona pellucida* were placed individually on 160 plants (fig. 3). The larvae were caught shortly before on the boundary of a field on which the oat crop had been totally infested by OSDV (experimental locality Sázava, Pelhřimov district).

The insects were divided into 16 groups of ten each, so that it was possible to carry out several variants of the experiment simultaneously. The individual variants differed as to the species of cereal on which the percentage of naturally infected larvae was determined, further as to the order in which larvae were caged on the cereals, the developmental stage of the cereal at the time of infection and the length of the test feeding on individual plants, which ranged from one to three weeks. The great majority of the leafhoppers in these experiments fed on five healthy cereals (of these at least three times on oats), some however survived for nine passages.

In the first phase of the experiments it was already shown that not all leafhoppers in populations from localities most strongly infested with OSDV were infected. Only about 86% of the individuals possessed the power of producing symptoms of disease. The remnant of about 14% of leafhoppers, although coming from a population of the same origin, never caused the changes characteristic of disease in oats, wheat or other cereals. Only after very thorough observation was it possible to note an extremely slight retardation of growth in plants on which they had fed. This became somewhat more marked when the period of sojourn of the leafhopper on the host plant was prolonged. This phenomenon, which is atypical for the disease, was usually noticable immediately after the application of the longest experimental period of test feeding

(three weeks) in plants at the earliest developmental stage (first leaf about 3 cm. long). However, after removal of the leafhoppers the majority of the plants concerned almost caught up with the unaffected control plants. This phenomenon might be due to the relatively great loss of assimilates caused by the intensive feeding by the leafhopper dependent on the young plant for its entire nutrition.

A certain proportion, always composed of the same insects (about 12% of the total), sometimes transmitted the striate virus as well as the sterile-dwarf. There were also some individuals among the experimental leafhoppers which exclusively transmitted the striate virus to the test plants.

In the majority of cases it was possible to distinguish the oat sterile-dwarf virus disease in oats with certainty only after four weeks from the time when the leafhoppers began to feed. Retardation of growth appeared a week earlier. In wheat, barley and rye dwarfing became evident approximately four weeks after infection; the final decision whether OSDV had really been successfully transmitted to wheat and barley was usually possible only after a further two to three weeks, when other symptoms of disease also appeared (stimulation of sprouting, retardation of earing, yellowing of the tips of the youngest leaves). In rye, which is clearly the most tolerant to OSDV, no symptoms were developed other than dwarfing. Differences in the damage caused by OSDV to individual cereals are to be seen in figs. 4, 5, 6 and 7.

According to Dlabola (1957, 1958) sterility and dwarfing in oats was caused only by larvae and females of the leafhopper *Calligypona pellucida*. The present experiments have shown that males transmitted OSDV to the same extent as larvae and females.

As a supplement to the experiments described the question was simultaneously investigated why in Dlabola's work (1957, 1958) using mass transfer of leafhoppers from areas not affected by the catastrophic occurrence of OSDV (the locality of Radotín near Prague) a similar picture of the disease was obtained on test plants as when insects taken from infested crops were used. In this investigation the authors were also concerned with the question of possible differences in the power of transmitting OSDV between populations of *Calligypona pellucida* from the Radotín area, where the disease was not known up to that time, and from Sázava, an epidemic focal point.

For this purpose 64 leafhoppers of the hibernating generation, caught on a meadow in the Berounka basin at Radotín, were individually caged on oat plants. It was found that something more than 6% of individuals in this locality were infected with OSDV. This finding points, above all, to a more general distribution of the virus. Further it makes it possible to explain Dlabola's observations as being due to a slight admixture of infected leafhoppers, which could easily distort the results when using his technique of mass caging of the insects on the test plants.

In a further phase of research an attempt was made to induce artificial infection of leafhoppers which had been shown to be uninfected over a month und half test period. In the Sázava population there were altogether 23 insects (cca 14%) which never caused infection with the sterile-dwarf or the striate viruses. By the end of May seven of them had died, however. The remainder, already as adults, were divided into two parts, one (control) was passaged on healthy plants, while an attempt to infect the other (experimental) was made as follows: three leafhoppers were isolated individually on sterile-dwarf oat plants, two on sterile-dwarf wheat plants and three on striate wheat for a period of one week.

The leafhoppers were then individually caged for test feeding on healthy plants in order to find out whether they had been infected. Eight transfers of such infected insects were carried out in all. The intervals of test feeding lasted three to ten days. At the end of the eighth passage (from July 14 to 18, 1958) no insect was still alive. In spite of this high mortality among the leafhoppers it was recorded that from the fourth test feed, when only four insects remained, two of them began to transmit OSDV regularly. Infectivity was acquired by one male, which had been infected on virus-infested sterile-dwarf wheat and one female, which had been infected on diseased oats. The circulation period of the virus in the bodies of these leafhoppers corresponded to an interval of 25 to 32 days. The remaining leafhoppers caused no infection to the end of their lives. The parallel testing of control leafhoppers did not infect healthy plants at all.

Similar experiments were carried out with a part of the leafhoppers from Radotín. In this case 23 adults, which in preliminary tests had shown themselves to be incapable of infection, were caged on virus-infected sterile-dwarf oats for acquisition feeding for nine days. They were then passaged as usual on to healthy plants until they died. In the first passage, which corresponded to a circulation period of 9 to 17 days in the body of the leafhopper, three out of

23 insects caused infection (cca 17%). The proportion of infectious leafhoppers then increased in spite of a sharply rising natural mortality, until in the last passage corresponding to a circulation period of 36 to 42 days it amounted to two-thirds (2 insects out of three survivors were infectious). The control insects, which were tested in parallel, did not infect the host oats at all.

In this way the first proof was provided that leafhoppers which were originally incapable of infection can acquire it if they are allowed to remain in contact with a source of sterile-dwarf virus. It was also possible to demonstrate that uninfected members of leafhopper populations both from epidemic areas and from other areas can act as vectors for OSDV. Probably viruliferous and non-viruliferous lines, such as those observed in the leafhopper *Cicadulina mbila* NAUDE, vector of *Zea virus* 2 (Storey) SMITH (v. e. g. KÖHLER and KLINKOWSKI 1954), do not exist in *Calligypona pellucida*.

Artificial infection of *Calligypona pellucida* larvae bred from eggs

In order to provide final proof of the infectiousness of the disease it was necessary to try to introduce a greater number of uninfected leafhoppers into the infection experiments. The easiest way of obtaining sterile leafhoppers was to breed them from eggs. This procedure could, however, only be successful on the assumption that it was a transovarially non-transmittable virus.

To this end gravid females from a highly infected population (80 to 90%) from Sázava were left to lay eggs (fig. 8) in stalks of healthy barley which was at the flowering stage and therefore practically immune from infection by OSDV. After three weeks the ripe straw with the eggs was cut off below the lowest nodes and placed in a flower-pot with young, just-sprouted oats in a silon isolator. The interval between the beginning of laying to the hatching of the first larvae was about 3 to 4 weeks. The whitish, very mobile larvae, which are difficult to see with the naked eye, were collected as soon as possible after hatching so that the theoretical possibility of their contamination by the dry barley straw should be reduced to a minimum.

The larvae so obtained were divided into several groups and simultaneous experiments were carried out with them in order to demonstrate the infective character of both diseases under investigation. More detailed mention will be made here only of the groups in which the infective character of OSDV was followed. The first group (control) was composed of 74 larvae, which were caged on healthy oats immediately on the first day after hatching. They were then transferred again to other cereals (mainly oats) at definite time intervals. The second group (experimental) contained a somewhat greater number of larvae, which were first placed together in a silon isolator for acquisition feeding on sterile-dwarf oat plants. The majority were left there for 13 days, some for a longer period. A total of 92 larvae was individually transferred to healthy oats after infection with the sterile-dwarf virus. After seven to twelve days of test feeding they were then in all cases transferred to new cereals.

The control larvae, which were never able to feed on diseased plants, were individually transferred during a period from the middle of July to the end of September to new plants (usually oats) ten times in all. No case of transmission of the sterile-dwarf virus occurred, although these larvae came from females infected to a high degree by the disease. The originator of sterility and dwarfing in oats is, therefore, not transferable transovarially.

On the other hand, however, from the third passage, i. e. from the time when the larvae were two to three weeks old, the typical signs of wheat striate virus disease began to appear in a varying percentage of plants. Throughout the series of passages it was observed that wheat striation was transmitted by about 12% of the leafhoppers and these were always the same individuals. This virus is, therefore, transovarially transmittable and it was possible to separate it in this way from the OSDV.

Quite different pathogenic characters are exhibited by larvae (experimental) which were left on sterile-dwarf oats. They infected 17% of the oats plants on which they fed with the sterile-dwarf virus immediately in the first passage after their transfer from the source of infection. In the majority of the remaining plants there was also a slight retardation of earing as compared with those fed on by larvae of the same population which had not, however, come into contact with plants suffering from the sterile-dwarf virus. In addition, about 10% of the plants in this experiment became diseased with the striate virus.

During the second passage of these larvae, which took place 12 days later and lasted 10 days, there was a 58·5% infection with the sterile-dwarf virus and in the further passage 81% of the

plants were infected. Although these experiments have not yet been completed, because they will be continued in 1959 following the period of vegetative passivity, there can be no doubt that the great majority of the larvae became infected and convincing proof has thus been provided that the originator of sterility and dwarfing in oats is infectious and therefore a virus. At the same time it was observed that the circulation period of the sterile-dwarf virus in the body of the vector is very variable and that in the majority of individuals it lasts for three to four weeks.

Determination of the time essential for leafhoppers to remain on host plants in order to infect them

Investigation of the time essential for leafhoppers to remain on healthy plants in order to infect them was carried out as follows: infected leafhoppers were individually caged on plants (10 to 15) for test feeding for 5 min., 30 min., 1 hour, $1^1/_2$ hours, 2 hours, 3 hours, 6 hours, 9 hours, 24 hours, 30 hours, 2 days, 3 days, 4 days and 6 days. The experiment was carried out twice.

Following five to ten minute sojourn of leafhoppers on oats no case of transfer of the sterile-dwarf virus was obtained. Isolated cases of infection were recorded following 30 minute isolation of the insects on healthy plants. The percentage of infection then rose sharply with increase in the length of sojourn on the plants, particularly in the intervals up to 30 hours, when 57% of the leafhoppers caused infection. After 3-day and longer periods all the insects transmitted infection.

Thus, in order for infection by one or both viruses to be achieved it was sufficient in principle for one leafhopper to stay for a relatively very short time on the test plant—the lowest limit being 30 minutes. It was found that the infected plant in general undergoes an equally severe form of the disease when it is infected by an insect feeding e. g. for one hour or for three weeks.

Investigation of the toxicity of extracts from homogenates of infected leafhoppers

The experimental procedure was as follows: 342 *Callipygona pellucida* adults (weight 0·77 g.) of a highly infected population from an epidemic area were cooled at 0° C to immobility. They were then quickly transferred into a cold mortar, 3 ml. of cold physiological solution for cold-blooded organisms (0·64% solution NaCl in water) was poured over them, some coarse-grained carborundum was added and they were thoroughly homogenized. The leaves of 121 oat plants in a silon frame were then mechanically inoculated with the liquid. The plants used were at the stage of one to two leaves.

After a week, when the experimental plants had reached a height of 12 to 15 cm., had three leaves and tiny, two to five cm. long shoots (still lacking nodes—first internodium), they were inoculated again; this time inoculation was carried out by injection. For this purpose 300 adults from a highly infected population were homogenized in a similar way in a porcelain mortar with 3 ml. of physiological solution for cold-blooded organisms. The homogenate was poured into small test-tubes for centrifuging, the mortar being washed out with a further ml. of physiological solution, which was added to the test-tubes. Following 3 mins. of centrifuging, at 4000 r. p. m. (g = 1608) the supernatant was further purified through a paper filter. The filtrate was immediately injected with a fine one-cubic syringe into oat stalks, the leaves of which had been mechanically inoculated the week before. Injections were made at regular intervals right through the stalk so that the excess liquid appeared in drops at both sides. Out of the 121 plants so treated 30 were treated in addition by the sediment formed during centrifuging being rubbed into the stalks by the carborundum technique.

All these operations were carried out rapidly and as far as possible in the cold (cooled vessels and physiological solution), so that the denaturation processes should be impeded to the maximum. It can therefore be assumed that relatively large quantities of the most various substances that can be extracted from leafhopper organisms were successfully introduced into the young plants in concentrated form. Oat plants of the same age and cultivated under the

same conditions as the experimental plants were used as controls and were inoculated only with pure physiological solution.

None of the 121 plants inoculated as described above developed symptoms of the viruses under investigation. They all grew normally in the same way as the control plants, came to ear and produced seeds.

This result is evidence of the fact that OSDV is not mechanically transferable. As regards the possibility that a toxin may be involved, it must be remembered that toxins are usually fairly stable substances and it is therefore unlikely that, using the quite careful method described, denaturation of such a substance could take place, if such a substance were indeed present.

Infection experiments using other methods than the agency of insect vectors

In 1958 new attempts were made, with negative results, to transfer cereal viruses through soil to which a considerable quantity of chopped straw and roots of sterile-dwarf oats and striate wheat had been added in the autumn of the previous year. Oats and wheat sown in this soil grew quite normally.

The attempt to transmit the viruses by means of the dodder (*Cuscuta campestris* YUNCK.) was not carried out on a sufficiently large scale for the results to be fully significant. Another reason for the lack of a positive result so far was evidently the fact that dodder fixes itself on to oats and wheat with great difficulty.

A successful experiment on the transfer of OSDV by an agent other than the leafhopper *Calligypona pellucida* may be considered to be that of grafting carried out by BLATTNÝ (1958), both by contact grafting in 1957 (v. PRŮŠA 1958b) and bottle grafting in the following year. Grafts from diseased plants as distinct from those from healthy plants produced a marked stimulation of sprouting and other symptoms of OSDV. This is a further proof of the virus character of this disease.

Investigation of the range of host plants of OSDV

Examination of the range of hosts of OSDV has so far been carried out only in pilot experiments. In the case of 14 grass species (*Lolium perenne* L., *L. italicum* A. BR., *Bromus inermis* LEYSS., *Phleum pratense* L., *Dactylis glomerata* L., *Avena fatua* L., *Agropyrum repens* P. B., *Setaria viridis* P. B., *Hordeum murinum* L., *Anthoxanthum odoratum* L., *Briza media* L., *Arrhenatherum avenaceum* P. B., *Poa annua* L., *Sieglingia decumbens* BERNH.) and two other plants (*Stellaria media* (L.) VILL., *Trifolium pratense* L.) observations were made whether they reacted to artificial infection and with what symptoms. Attempts at reverse transfer were not made. Infection was carried out by the caging of groups of 10 to 20 leafhoppers on about the same number of young plants. Although it was later ascertained that only a small percentage of the leafhoppers used (from Čejkov, Pelhřimov district) was infected with the oat sterile-dwarf virus, the characteristic symptoms were caused in two grasses, *Avena fatua* L. and *Poa annua* L. (figs 9 and 10). *Avena fatua* seemed to be more strongly affected by the oat sterile-dwarf virus than was the very susceptible cultivated variety of oats, Valečovský bílý, on which all experiments had been made. Both these grasses were also successfully infected with the wheat striate virus.

This result indicates that in studying the epidemiology of both viruses we should not underestimate the part which may be played by some grasses, and perhaps other plant species, as sources and particularly as reservoirs of infection in nature.

Discussion

It was ascertained in Finland that much greater damage is caused to oat crops by the mass transfer of leafhoppers *Calligypona pellucida* from areas of epidemic oat disease than by those caught in districts where the disease

was not widespread (Heikinheimo 1957; Kanervo et al. 1957). The Finnish authors evolved two hypotheses from their results as to the actual cause of the disease: either a new virus is involved, or two population strains must exist among leafhoppers differing in the toxicity of their salivary excretions (Kanervo et al. 1957). Dlabola (1957, 1958), who worked parallel with the present authors, also assumed that the cause of the disease might be either of the above factors, i. e. toxin or virus. Nuorteva (1958) rejected—without proof— possibility of a virus being the cause and maintained that it is a toxin. This author tried to investigate the effect of extracts of the crushed salivary glands of *Calligypona pellucida* on the growth of cuttings from etiolated bean shoots, using the method which is otherwise employed for evaluation of the phytotoxicity or phytostimulation of insecticides (Casida and Allen 1951a, 1951b).

So far, however, no one, either in Finland or in Czechoslovakia, has succeeded in obtaining convincing proof to justify the toxin hypothesis. The mass transfer of leafhoppers to test crops, which has so far been the only method employed in infection experiments with OSDV, was incapable of providing a solution regarding the existence of toxic and non-toxic strains because it is not possible in this way to determine whether the disease has been caused by all or only some of the leafhoppers concerned. On the other hand, the individual study of the pathogenic powers of single insects on the plants enabled the authors of the present paper to separate individuals which caused disease in the plants on which they fed from those which did not influence the health of the plants on which they fed. Non-infectious individuals, both from OSDV epidemic areas and from areas where the occurrence of the disease was unknown, were then successfully infected by artificial means.

The results of this work, which have been sufficient to exclude the assumption that the cause of the symptoms under investigation is a non-infectious toxic substance, can be supplemented by the following findings.

It was observed that about four weeks elapse from the time when infectious leafhoppers are caged on oat plants to the time when it is possible to record reliable symptoms of OSDV. Neither local lesions nor necroses develop. The incubation period in other plants is still longer. Only after a considerable incubation period does a clear picture of systemic disease appear. For example, oats takes on a darker green colouring, the youngest internodes do not lengthen, the bases of the stalks swell, sprouting increases, etc. The systemic character of the disease is also illustrated by the following experiment: after oats had come to ear the main stalk was removed above the first node in 60 plants suffering from OSDV and in 60 healthy plants. The plants were then richly manured with nitrogenous and phosphatic manures and adequately watered. While shoots of the healthy plants developed normally and in most cases formed ears, those of the diseased plants remained dwarfed to the end of the vegetative period, although their number increased several fold as compared with the healthy plants.

If the originator of the disease is a toxic substance injected by the leafhoppers it must exert a systemic effect, which is extremely rare. If, however, the existence of systemic action by a toxin were nevertheless to be assumed

we should come into conflict with the pathogenesis of the disease. The disease would necessarily grow worse proportionately to the length of the feeding time of the insect on the plant. This does not, however, happen. Further, injury by a toxin should be most evident in the host just at the time when the insect is feeding, or shortly after its removal from the plant. Then the plants should recover more or less quickly. Nothing of the sort, however, occurs. The disease acts quite to the contrary, for its symptoms appear and then show a sharp progressive intensification quite a long time after the infected leafhoppers have fed on the plants.

The course of the disease clearly indicates that its originator reproduces itself in the host plant, because as long as it does not attain a certain accumulation the plant does not react to its presence with the characteristic symptoms. It is significant that the length of the incubation period during which this reproduction takes place is practically independent of the length of the feeding time (hours or weeks) of the infected insects on the plants. A plant can develop the most severe form of the disease quite independently of the number of insects caged on it. Feeding by a single infected leafhopper, which may last in some cases for a very short time (30 min.) is quite sufficient to cause infection. These facts are certainly in direct contradiction to the toxin hypothesis.

The fact that two different diseases have been found to be spread by the same species of leafhopper is also in itself a serious argument against the theory that symptoms of disease could be caused by toxins. If this were to be the case it would be necessary to assume that one insect species, homogeneous both in origin and morphology, must possess four physiologically differing populations, i. e.: non-toxic, with toxin producing the sterile-dwarf disease, with toxin producing the striate disease and finally with both toxins. Anything of the kind is most improbable.

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Вирусная стерильность и карликовость овса

ВЛАДИМИР ПРУША, ЕВГЕНИЙ ЕРМОЛЬЕВ, ИОЗЕФ ВАЦКЕ

Резюме

В статье даются новые доказательства, окончательно подтверждающие вирусный характер стерильности и карликовости овса (ВСКО), эпидемия которой была в 1956 г. в Йиглавском крае.

Приводятся замечания об обнаружении другого вируса желтухи, который в отличие от ВСКО вызывает заметную полосатость у хлебных злаков и, повидимому, идентичен с вирусом, описанным недавно (*Slykhuis* 1958) в Англии, и, может быть, также с вирусом, наблюдаемым в Германии и Дании под названием European cereal striate mosaic.

Вирусная полосатость пшеницы (ВПП), как мы называем эту болезнь, интересна в теоретическом отношении, так как зараженные самки *Calligypona pellucida* F. могут передавать ее потомству трансовариально; это свойство было впервые доказано у фитопатогенных вирусов на европейском континенте. На этом основании стало возможным разделить оба вируса, так как ВСКО трансовариально не переносится.

В статье даны доказательства того, что у цикадки *Calligypona pellucida* F. не существуют две генетически и физиологически отличающиеся популяции, существенно отличные токсичностью своих слюнных выделений. Гипотеза о существовании таких популяций возникла в Финляндии и ее сторонники имелись также в ЧСР.

Оба вируса являются первыми обнаруженными на хлебных злаках в ЧСР вообще. У обоих заболеваний переносчиками являются цикадки *Calligypona pellucida* F. ВСКО может переносится как личинками так и имаго. Продолжительность циркуляции ВСКО в теле переносчика довольно варьирует (от 9 до 42 дней), но в большинстве случаев не превышает 3—4 недель.

Инкубационный период ВСКО длится у овса 3—4 недели, у пшеницы и ячменя 4—7 недель; рожь отстает в развитии примерно 4 недели после инфекции, однако другие болезненные признаки у нее не проявляются. ВСКО более разрушительно действует на овес, чем на пшеницу или ячмень, на рожь действует меньше всего.

Для переноса ВСКО достаточно и получасовое пребывание одной зараженной цикадки на восприимчивом растении. После 3-дневного нахождения зараженной цикадки на овсе всегда имело место заражение вирусом.

Распространение ВСКО не ограничивается одной только областью эпидемии, так как наличие вируса удалось экспериментально доказать в гораздо меньшем масштабе в одном из местонахождений вблизи Праги.

Степень разрушительности ВСКО не увеличивается пропорционально продолжительности сосания цикадки на растении. Поэтому увеличение количества цикадок на растении не может иметь значение для степени повреждения растения, так-как уже одна цикадка способна перенести заболевание с самыми тяжелыми последствиями.

Концентрированные экстракты из гомогенатов тел зараженных цикадок, применявшиеся в избытке, как путем механического втирания в растения, так и путем инъекции в стебли, никогда не вызывали заметного повреждения у овса. Опыт с переносом ВСКО и ВПП почвой дал отрицательный результат. Так же опыт с переносом обоих вирусов через повилику (*Cuscuta campestris* Yunsk.) был с отрицательным результатом, хотя в этом случае это могло быть связано с тем, что повилика не склонна к паразитизму на хлебных злаках.

Единственным успешным опытом по переносу ВСКО иным способом, чем через насекомое, мы считаем результат опыта с прививкой (Blattný 1958).

При предварительном исследовании ареала растений-хозяев в природе было обнаружено, что оба вируса хлебных злаков могут вызывать характерные симптомы у *Avena fatua* L. и *Poa annua* L.

В дискуссии анализируются некоторые дальнейшие факты с точки зрения этиологии.

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Fig. 1. Cages of brass wire and silon thread used for the individual infection of cereals.

Fig. 2. The technique of individual infection: in each pot some of the plants were left uninfected as controls.

Fig. 3. Larvae of the leafhopper *Calligypona pellucida* F.

Fig. 4. Valečovský bílý oats infected with the sterile-dwarf virus at the stage of one leaf; the healthy control plant (left) is just coming to ear.

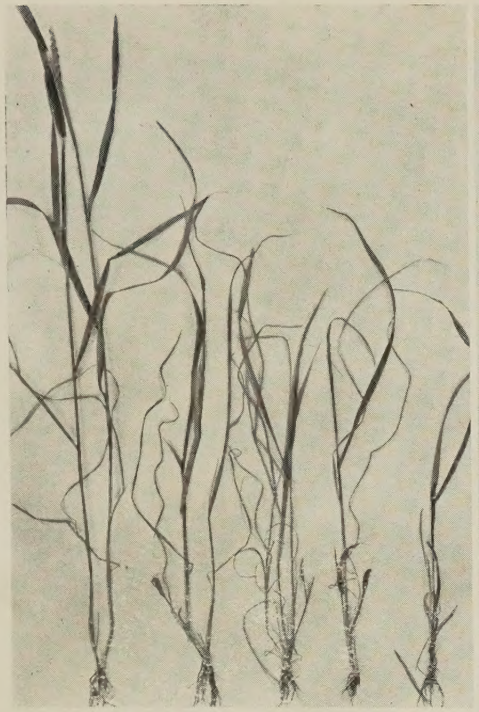

Fig. 5.

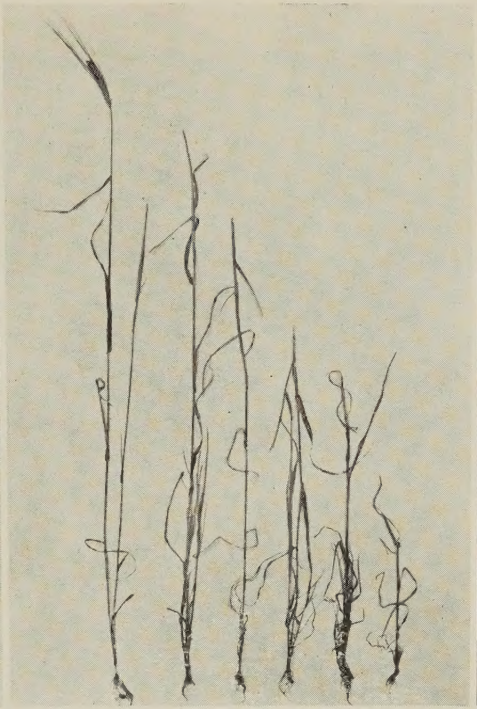

Fig. 6.

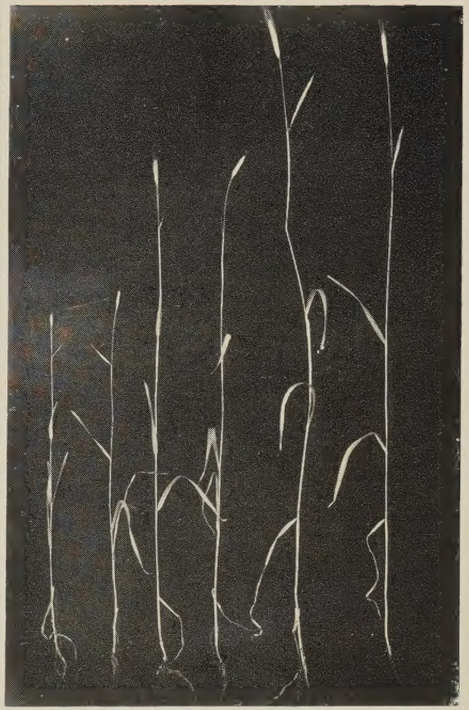

Fig. 7.

Fig. 5. Spring wheat (var. Ratbořská) infected with sterile-dwarf virus at the stage of one leaf; the uninfected plant (left) is just coming to ear.

Fig. 6. Spring barley (Stupický hanácký) infected with sterile-dwarf virus at the stage of one to two leaves; uninfected control plant on the left.

Fig. 7. Spring rye (Těšovské) infected with sterile-dwarf virus at the stage of one leaf; two healthy plants to the right.

Fig. 8.

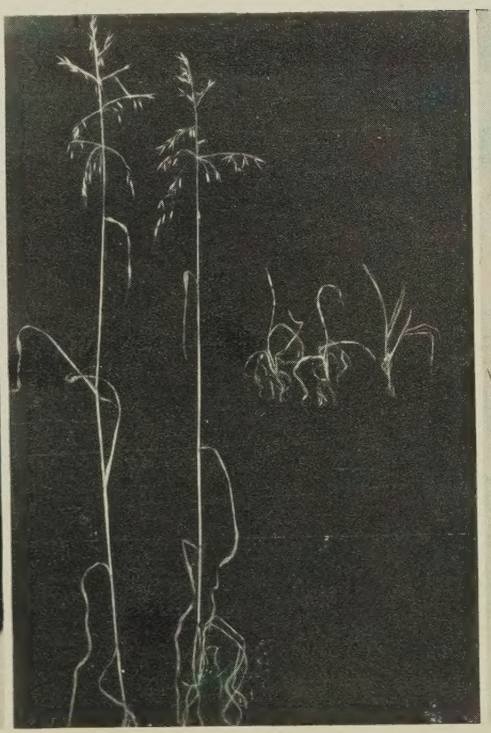

Fig. 10.

Fig. 9.

Fig. 8. Eggs of the leafhopper *Calligypona pellucida* in a split barley stalk.
Fig. 9. OSDV in *Avena fatua* L.; two healthy plants to the left.
Fig. 10. OSDV in *Poa annua* L.; the plant on the left is healthy.

biologia plantarum

Časopis pro experimentální botaniku
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